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AN EXAMPLE OF THE ABILITY OF RIBES LACUSTRE TO INTENSIFY CRONARTIUM RIBICOLA ON PINUS MONTICOLA[1]

By J. L. MIELKE[2]

Assistant pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Four species of *Ribes*[3] are of special importance in the control of white-pine blister rust (*Cronartium ribicola* Fisch.) in the commercial range of western white pine (*Pinus monticola* Dougl.) in northern Idaho, northeastern Washington, and western Montana because of their abundance, distribution, and reaction to the rust (*12*).[4] These, in the order of their numerical prominence, are *Ribes lacustre* (Pers.) Poir., *R. viscosissimum* Pursh, *R. inerme* Rydb., and *R. petiolare* Dougl.

Extensive field studies in the rust-infected regions of western North America have shown that very wide differences exist among these four species both as to their susceptibility to the rust and to the subsequent development of telia (*9*).[5] Their order of prominence in these respects is: *Ribes petiolare*, *R. inerme*, *R. viscosissimum*, and *R. lacustre*, the first two species being relatively very high, the third medium to low, and the last very low. On the basis of the high susceptibility of and profuse telial development on *R. petiolare* and *R. inerme*, as observed early in the course of field studies in British Columbia (*3*), it was obvious that rapid and severe damage to the associated stands of western white pine (*Pinus monticola* Dougl.) would occur as the rust spread into the region centering in northern Idaho. Both *R. petiolare* and *R. inerme* are confined by habitat requirements to limited areas along stream courses, which are favorable sites for the development of the fungus. Therefore, in the region under discussion, the large-scale protection work against the rust carried on by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, had as its immediate objective the systematic eradication of ribes in the stream-type areas where one or more of these highly susceptible species occurred (*13*).

As the rust spread and intensified in the region comprising northern

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[2] The author is indebted to H. G. Lachmund, formerly pathologist, Division of Forest Pathology, who supervised the study, and to A. A. McCready, W. F. Cummins, and C. J. Nusbaum, former members, and J. W. Kimmey, T. S. Buchanan, and T. W. Childs, present members, of the same Division, for assistance in the collection of the data on which this paper is based. Acknowledgment is also made to G. B. Posey, formerly of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for advice and suggestions during the course of the study, to Dr. H. T. Güssow, Dominion botanist, and to various other members of the Dominion and Provincial Governments of Canada for their friendly cooperation.

[3] The genus name *Ribes*, as well as the common noun "ribes," is used in this paper to include both currants and gooseberries.

[4] Reference is made by number (italic) to Literature Cited p. 882.

[5] KIMMEY, J. W. SUSCEPTIBILITY OF RIBES TO CRONARTIUM RIBICOLA IN THE WEST. Jour. Forestry [In press.]

Idaho, western Montana, and northeastern Washington, continued observations showed that pine infection was developing in damaging proportions in upland areas where both *Ribes viscosissimum* and *R. lacustre* were present. Recognition of this condition made it necessary for the control program in this region to include the eradication of these species over the upland areas as well as the more susceptible species found only in the stream-type areas. Since, however, *R. lacustre* almost always occurs in association with other *Ribes* species, practically nothing was known regarding its pine-infecting capacity, but, on the basis of previous knowledge of its reaction to the rust, together with observations in the field, the species was thought to be relatively innocuous. The present study was undertaken to test this assumption.

This paper reports the results to date of a study made over a period of several years in the vicinity of Revelstoke, British Columbia, on a plot situated within the only known area in western North America where the prickly currant (*Ribes lacustre*) has been solely responsible for blister rust infection of a stand of western white pine. Because the Revelstoke area is well within the range of *Pinus monticola*, results of the study are considered of value in connection with control of the rust in the region centering in northern Idaho.

LOCATION AND DESCRIPTION OF THE STUDY PLOT

The plot is located in the interior of British Columbia, approximately 9 miles north of Revelstoke and on the east side of the Columbia River. It is 45 acres in size and is situated within the edge of an old burn on a steep mountainside with a westerly exposure. A few small creeks, the largest of which enters the plot near the southeast corner and runs out midway of the western boundary after forking several times, drain the area very well (fig. 1). A large rockslide near the northeast corner considerably reduces the density of vegetation in that part.

Bordering the plot on the south side is a stand of mature timber, composed mainly of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western red cedar or giant arborvitae (*Thuja plicata* D. Don), and Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britton), which extends southward for about 6 miles. To the north and east is an extensive burned area on which there occurs a sparsely stocked stand of reproduction composed mainly of western white pine and aspen (*Populus tremuloides* Michx.). Near the west boundary is the Columbia River, and on the opposite side of it there is a very extensive stand of mature timber composed mainly of western hemlock, western red cedar, and western white pine.

The only pine, and also the predominant tree species, on the plot is *Pinus monticola*. All species are unevenly distributed on the area, with the greatest density of growth on the southwest half. At first glance the stand appears to be almost pure pine in its composition, and in this regard it is typical of many others in the region. The principal tree associates of the pine are alder (*Alnus sinuata* (Regel) Rydb.), willows (*Salix* spp.), paper birch (*Betula papyrifera* var. *subcordata* (Rydb.) Sarg.), aspen, Douglas fir, western hemlock, and western red cedar. The stand is in the reproduction stage and was about 25 years of age at the time the study was started. The pines averaged slightly over 12 feet in height.

Bushes of *Ribes lacustre*, the only *Ribes* species present, are concentrated along the creeks and scattered elsewhere on the plot (fig. 1). This same species was also noted in moderate abundance along streams and on moist areas on the lands surrounding the plot, particularly to the south, east, and west. No other *Ribes* species were found closer than about 8 miles distant.

FIELD PROCEDURE

The area was located in the late fall of 1927, but no work was done on it that year. In 1928 the plot boundaries were surveyed and the 45-acre area within them was mapped and subdivided into blocks 1 chain square (fig. 1). Numbered stakes were placed at all block corners to facilitate future work.

In the summer of 1929, all the *Pinus monticola* trees on the plot were numbered, and their positions, together with the positions of all *Ribes lacustre* bushes, were mapped. Also, the number of feet of live stem for each ribes bush was obtained for use in computing the average amount of live stem per acre. No general survey of pine infection was made, but all blister rust cankers observed during the course of the other work were tagged and significant data recorded. Systematic examinations were made, however, of 100 bushes of *R. lacustre* to determine the amount of rust present on them. These bushes, which mainly occurred scattered over that portion of the plot having the greatest density of

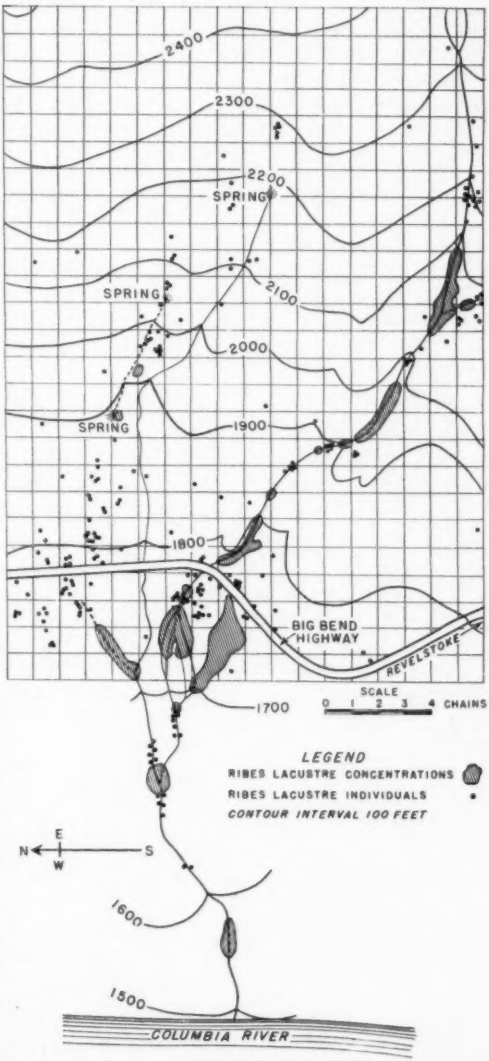

FIGURE 1.—Revelstoke natural pine infection area plot, Revelstoke, British Columbia.

the ribes and the pines, were tagged and used in subsequent years for the purpose of measuring annual infection. On the basis of the author's past experience with the rust, the 100 bushes were considered more than an adequate number for giving an index of the degree of infection over the entire plot. Also, extensive observations indicated that the infection on them was as great, if not slightly greater, in amount than would be the average for all bushes.

The systematic examination of the pines for blister rust infections was started in mid-June 1930. A numbered tag was attached to each canker found in order to prevent duplication and for future reference. Recorded data included: (1) Location on the tree of the infected branch, (2) diameter of the branch at the lower limits of the canker, (3) distance from the lower limits of the canker to the bole, (4) diameter of the bole at junction of the infected branch, (5) age of growth on which the canker originated, and (6) stage of development of the canker. Using these data, together with information on canker growth and effects reported by Lachmund (*6*), experienced assistants made estimates in the field as to whether the fungus would grow down the branch and into the bole. In the event that this was expected to happen, the number of years required for such growth and for the consequent death of or severe damage to the tree was also estimated. Limited time and personnel permitted examination of approximately only 60 percent of the trees in 1930, the remainder of them being examined during the following season.

The data available by the fall of 1931 showed that practically all pine infection on the plot occurred during the years 1922 to 1928, inclusive, the greatest part of it having originated in 1927. Any infection that might have taken place subsequent to 1928 was not yet readily apparent. Observations made in the fall of 1931 and spring of 1932, however, revealed that numerous cankers had not been found during the course of the examination in 1930, apparently mainly because of their small size at that time. Consequently, all the pines were reexamined in the summer of 1932, at which time many additional cankers of 1927 origin and some of 1928 origin were found. All of these were tagged and the usual data on them were obtained and recorded. Since the reexamination was being made 5 years after the heavy wave of infection in 1927 and 4 years after a much lighter wave in 1928, it was concluded, on the basis of earlier studies on canker growth and development (*4*, *5*, *6*), that all cankers having their inception in those years and previously should be of sufficient size to be readily visible in 1932. A check made a few years later indicated that this conclusion was correct. However, it was also found that a small percentage of cankers had still escaped detection during the reexamination. Failure to find these few was not ascribed to their small size but to the human factor involved.

RIBES INFECTION

No records of the extent of blister rust on *Ribes lacustre* are available for 1922, the year of first-known pine infection, nor were systematic data obtained for 1927, the year of greatest intensification of the disease on pine. The first examination of *R. lacustre* was but a cursory inspection of about 30 bushes made in the fall of 1927 at the time the area was discovered. Less than half of them bore evidence of

the rust, which was present only in slight amount. In the fall of 1928 an intensive survey was made of this host on the plot, and again the rust was found to be very sparse, the degree of infection being less than half that recorded in any subsequent year. Starting in 1929, however, systematic data were obtained annually until 1935 on the 100 tagged bushes previously mentioned. These data, which are summarized in table 1, were recorded and computed by the same methods described in a previous paper (*9*).

From table 1 it is evident, even for those years in which there occurred the greatest development of the rust on *Ribes lacustre*, that while only an extremely small percentage of the total lower leaf area became infected and a still smaller percentage bore telia there has been an almost consistent annual increase in the amount of rust. From these records, together with those obtained in 1927 and 1928, it would seem possible that a similar relationship might have extended back to 1922. If this relationship did not extend back to 1922, it still is reasonably safe to assume that the fungus was never much more abundant on *R. lacustre* than in those years for which data are available, for, as is later shown herein, very few sporulating cankers could have been present on the area to have caused such infection. Even if the rust was more prevalent than that shown from 1929 to 1934, however, it seems doubtful if it ever was present in sufficient amount to be classed as a heavy attack on this *Ribes* species. In support of these suppositions it may be demonstrated that the recorded infection has been very light when percentages of total leaf area infected and total leaf area bearing telia (table 1) are compared with the average figures for susceptibility and telium-producing capacity of *R. lacustre* presented in table 2. The figures in table 2 were obtained by Mielke, Childs, and Lachmund in their large-scale relative-susceptibility studies (*9*). Since nearly all the *R. lacustre* bushes on the Revelstoke plot grew in the shade or partial shade of other vegetation, the data presented for them should be compared mainly with the data for corresponding growth forms shown in table 2.

TABLE 1.—*Annual blister rust infection of 100 tagged Ribes lacustre bushes on the Revelstoke plot*

Year	Bushes		Total leaves		Total lower leaf area	
	Examined	Infected	Examined	Infected	Infected	Bearing telia
	Number	*Number*	*Number*	*Number*	*Percent*	*Percent*
1929	100	39	76,585	738	0.05	0.01
1930	100	93	59,650	837	.07	.03
1931	100	100	56,710	1,815	.19	.05
1932	100	100	36,745	5,144	.59	.17
1933	100	100	57,415	6,316	.33	.07
1934	98	98	65,965	6,597	.35	.04

TABLE 2.—*Average figures for susceptibility and telium-producing capacity of Ribes lacustre*

Form of *R. lacustre* as related to exposure to sunlight	Total lower leaf area	
	Infected	Bearing telia
	Percent	*Percent*
Open	3.4	0.4
Part-shade	4.3	.7
Shade	8.1	2.9

PINE INFECTION

As a result of the examination of the pines made during the years 1929 to 1932, inclusive, a total of 10,109 cankers was recorded. Analysis of the data, by Lachmund's (*4*) method of determining the age of blister rust infection on western white pine, indicated that 337 of these cankers had their inception in 1922, 150 in 1924 and 1925, and the remainder, 9,622, in 1927 and 1928. Possible infections originating in 1929 or subsequently had not yet reached a readily visible stage of development at the time of the last examination in 1932, and consequently no particular effort was made to record them. Therefore, only those cankers that had their inception in the years 1922 to 1928, inclusive, are considered in the study.

Neither the exact place at which pine infection first became established on the area nor any original canker was discovered. As a result of extensive studies on the ground, however, it was evident that both the place of origin and the center of pine infection were well within the plot boundaries. Of the oldest cankers found 337 were on the plot and a few others in the immediate vicinity, all of which had their inception in 1922, but that year is not regarded as the one in which pine infection originally occurred on the area. According to the author's experience in the West, the abundance and scattered distribution of these cankers would strongly indicate that a few older ones must have been present at one time, for almost invariably the number of trees originally infected and the number of original cankers at an infection center are comparatively small, being generally less than 10 even when highly susceptible ribes species are present. In this case, over 300 widely distributed pines bore the cankers that originated in 1922, and consequently it would seem reasonable to assume that some pine infection must have occurred prior to that time. Considering the available evidence, together with the normal development of the fungus (*5*) and the general spread in 1917 and 1918 (*3*), it is believed that the rust first became established in pines on the plot in either one of those years. Failure to find any older cankers may very possibly be attributed to destruction of the tree or trees that bore them when a highway (fig. 1) was built through the area about 1925, for this highway crosses the part of the plot that has the greatest density of pine infections.

The results of the studies on pines are summarized in table 3. Observations made in 1933 indicated that possibly as many as 5 percent or more of the cankers had been missed during the examinations, consequently the number of trees infected, the number of cankers, and the degree of damage were somewhat greater than the corresponding data shown in the table. Since, as previously stated in the section on Field Procedure, the data on tree damage are based mainly on estimates of future development of the fungus in the bark, their reliability may possibly be questioned. All cankers found were tagged, however, and it was therefore feasible to make a future check of the estimates. This was done in the fall of 1936, five to seven seasons later, depending upon the time the original work was done. Results of the check indicated that the estimates were of sufficient accuracy to make revision of the summary data unnecessary. From the data in table 3, it is anticipated that a higher percentage of trees infected in 1922 will be killed or damaged than of the trees infected during the entire period of 1922–28. A discussion of the factors

responsible for this percentage difference is not considered within the province of this paper, but they are known to include height of tree, width of crown, and difference in basis of trees infected in the different years.

TABLE 3.—*Blister rust on western white pine on the Revelstroke plot*

Years in which infection occurred	Trees						Cankers		
	Total on plot	Infected		To be killed or damaged [1]			Total	Average per tree on basis of—	
				Number	Percentage of infected trees	Percentage of total trees		Infected trees	Total trees
	Number	*Number*	*Percent*				*Number*	*Number*	*Number*
1922	9,316	312	3.35	203	65.06	2.18	337	1.08	0.036
1922 to 1928, inclusive	[2] 9,920	3,417	34.45	1,600	46.82	16.13	10,109	2.96	1.02

[1] The term "damage", as used in this paper, denotes injury to the pines to the extent of preventing ultimate merchantable value.
[2] The difference in total number of trees is accounted for by the increase in number of seedlings.

Although it is evident that the intensification up to 1928, inclusive, has been very rapid, this cannot be considered as exceptional, for instances of similar and even greater rates of increase in the rust on *Pinus monticola* have been reported (*7; 8, p. 303; 11*), and the author has observed many others during the past 12 years. If the rust continues to increase at the same rate that it did from 1922 to 1928 (table 3), nearly all pines on the plot will be infected by approximately 1940. An examination of the weather records for Revelstoke indicated that both 1922 and 1927 were favorable years for the infection of pines, whereas the intervening years and 1928 were relatively unfavorable. Approximately only one season in every 5 years has been favorable for general spread of the rust in the interior region, while in the coastal region of British Columbia, Pennington (*10*) found this average to be one in every 4 years. Accordingly, it would appear that intensification of the fungus on the plot will be governed largely by climatic conditions and will occur at an irregular rate. A reliable estimate of the time required for infection of all pines is therefore difficult to make.

The number of feet of live stem per acre is commonly used as a measure of the abundance of a *Ribes* species on an area. It seemed desirable, therefore, to use this criterion in determining the amount of *Ribes lacustre* on the plot. As the result of a careful check, a total of 24,111 feet, or an average of 536 feet per acre, was found. The bushes, which varied greatly in size, were not generally distributed over the area, but occurred in greatest density in the vicinity of the creeks (fig. 1). *R. lacustre* was also found in the immediate vicinity of the plot, but in lesser densities, and because of their location these bushes are not regarded as being responsible for many of the pine infections. Whether occurring on or off the plot, however, *R. lacustre* was solely involved, for it is the only species of *Ribes* known for several miles around. There are plantings of the cultivated black currant (*R. nigrum* L.) about 8 miles distant, but spores from these plants are not believed to have caused any of the cankers on the plot, for no instances

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are known of spread from *Ribes* to pines over distances even one-third as great.

The pines occur in greatest density on the south half of the plot, and except for a few small areas the stand cannot be considered as fully stocked, for in 1927 there was an average of only about 220 trees per acre. The older trees have been bearing cones for several years, and some seedlings appear annually. However, if the present rate of intensification of the rust and the small number of cankers required to kill such young trees are considered, few of them may be expected to survive for very long. Accordingly, there appears no possibility of the area becoming fully stocked with white pine or of the present stand reaching maturity so long as *Ribes lacustre* is present.

DISCUSSION

Western white pine occurs in two separate belts, which may be designated as coastal and interior. Both the Revelstoke area in British Columbia and the region centering in northern Idaho are within the interior belt. The same tree species occur over most of this region, and the summer climate is fairly uniform, although a somewhat greater amount of precipitation occurs at Revelstoke than in northern Idaho. This similarity in general conditions between the two places would seem to make the results of the study of more than local application. Before any final conclusions are reached, however, certain climatic factors should be more carefully considered, for weather conditions to a large extent determine the spread and severity of blister rust. According to Pennington (*10*), moisture is more important than temperature for infection of pines and ribes. Because of the importance of moisture, a comparative summary of the rainfall during the growing season at Revelstoke, the weather station nearest the plot, and various places in north Idaho is given in figure 2. It is evident from the pine-infection data that *Ribes lacustre* caused rapid intensification of the rust on *Pinus monticola* near Revelstoke, but rainfall there is somewhat more abundant than at the weather stations in Idaho used for comparison. This difference in rainfall is apparently of little consequence, however, for the author has been unable to detect its effect in any difference in the development of the rust between the two regions, and according to various reports (*1*, *2*, *11*, *12*, *13*, *14*), the fungus is well established and intensifying at a very rapid rate in the white pine forest of Idaho. The results of the study would therefore appear to be of value in connection with control of the rust in the region centering in northern Idaho.

The rapid intensification of the rust that occurred on the plot was contrary to expectation, for it was thought that *Ribes lacustre*, with its low susceptibility and low telium-producing capacity, would not cause such abundant infection of pines. The data strongly indicate, however, that *R. lacustre* constitutes a far greater menace than has hitherto been assumed and that susceptibility and capacity to produce telia are not necessarily safe indexes of the relative importance of a *Ribes* species as a source for pine infection. The results of the study are of special significance in illustrating that stands of western white pine in infected areas may be severely damaged by blister rust if *R. lacustre*, which is the least susceptible of the four most important species in the pine region centering in northern Idaho, is allowed to remain in considerable numbers within and adjacent to the stands.

Aside from the fact that *Ribes lacustre* is capable of rapidly intensifying blister rust on western white pine, there are other factors that make it a very important host in the region centering in northern Idaho. Numerically it is the most abundant of the four common ribes in that region, as evidenced by the control work from 1923 to 1935, inclusive, for nearly one-half the total number of ribes destroyed were of that species.[6] *R. lacustre* is widely distributed over the region, occurring generally in the forested area. It is a tolerant species and is not confined to any particular site conditions, growing not only in moist soils bordering streams but also on the drier upper slopes. The wholesale destruction of this plant by chemical means has not yet been practicable, partly because of its resistance to ordinary dosages of chemicals that may be safely or economically used. Since *R. lacustre* is a species that propagates readily by layering, hand eradication is somewhat more difficult than with some of the other ribes. It would seem, therefore, that *R. lacustre* is as much if not more of a problem in the control of the rust than some of the more susceptible species.

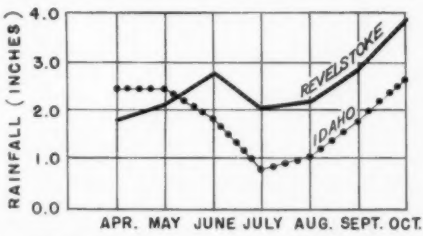

FIGURE 2.—Graphical summary of rainfall at Revelstoke, British Columbia, and in Idaho. The monthly rainfall averages for Revelstoke cover a period of 32 years and were taken from British Columbia Department of Agriculture weather records, Report for 1933. The averages for Idaho were computed from United States Weather Bureau records obtained at Avery, Prichard, St. Maries, Priest River Experiment Station, and Pierce, covering various periods ranging from 16 to 32 years.

SUMMARY

Four species of *Ribes* are of special importance in the control of white-pine blister rust (*Cronartium ribicola*) in the western white pine region centering in northern Idaho. Numerically, the most prominent of these is the prickly currant (*Ribes lacustre*), which is relatively very low both in susceptibility to the rust and in the production of telia. This paper reports the results of a study, conducted over a number of years on a 45-acre plot near Revelstoke, British Columbia, on the ability of this currant to spread blister rust to western white pine (*Pinus monticola*).

Although the known infection of *Ribes lacustre* on the area has been relatively small in amount for this species, the rust has intensified rapidly on the pines. The available evidence indicates that a few trees first became infected in either 1917 or 1918. In 1922 over 3 percent were infected and by the end of 1928 about 34½ percent of the total of 9,920 pines on the plot were diseased. The cankers increased in number from 337 in 1922 to 10,109 in 1928. This attack has been severe enough to result in the death or serious damage of 1,600 trees, or over 16 percent of the total.

If a similar rate of intensification of the rust continues, it will obviously be but a matter of a few years before practically all the pines become infected and serious damage or death results. There appears no possibility that the stand of western white pine on the area will reach maturity under the prevailing rust conditions.

Results of the study indicate that *Ribes lacustre* constitutes a far greater menace to western white pine than has hitherto been assumed

[6] Information furnished by officials of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, and used here with their permission.

and that this species must be eradicated where it occurs in or near pine stands if such stands are to be protected against the rust.

LITERATURE CITED

(1) Detwiler, S. B.
1931. The white pine blister rust situation. Jour. Forestry 29: 181–185.

(2) ———
1933. General aspects of the white pine blister rust situation. Jour. Forestry 31: 514–521.

(3) Lachmund, H. G.
1926. Studies of white pine blister rust in the west. Jour. Forestry 24: 874–884.

(4) ———
1933. Method of determining age of blister rust infection on western white pine. Jour. Agr. Research 46: 675–693, illus.

(5) ———
1933. Mode of entrance and periods in the life cycle of Cronartium ribicola on Pinus monticola. Jour. Agr. Research 47: 791–805, illus.

(6) ———
1934. Growth and injurious effects of Cronartium ribicola cankers on Pinus monticola. Jour. Agr. Research 48: 475–503, illus.

(7) ———
1934. Damage to Pinus monticola by Cronartium ribicola at Garibaldi, British Columbia. Jour. Agr. Research 49: 239–249, illus.

(8) Mielke, J. L.
1933. Tuberculina maxima in western North America. Phytopathology 23: 299–305.

(9) ——— Childs, T. W., and Lachmund, H. G.
1937. Susceptibility to Cronartium ribicola of the four principal Ribes species found within the commercial range of Pinus monticola. Jour. Agr. Research 55: 317–346, illus.

(10) Pennington, L. H.
1925. Relation of weather conditions to the spread of white pine blister rust in the Pacific Northwest. Jour. Agr. Research 30: 593–607, illus.

(11) Putnam, H. N.
1931. Spread and development of white pine blister rust in the inland empire. Northwest Sci. 5 (3): 53–58.

(12) United States Bureau of Entomology and Plant Quarantine.
1935. White pine blister rust control. U. S. Bur. Ent. and Plant Quarantine Rept. of Chief. 1935: 34–36.

(13) Wyckoff, S. N.
1930. Blister rust control in the inland empire. Timberman 31 (8): 162, 164, 166, 168.

(14) ———
1934. The white pine blister rust situation. Calif. Dept. Agr. Monthly Bull. 23: 296–297.

WATER SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE BLACKFIRE DISEASE OF TOBACCO[1]

By E. E. CLAYTON

Senior pathologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In a previous publication [2] the general situation with respect to the wildfire and the blackfire or angular leaf spot diseases of tobacco (*Nicotiana tabacum* L.) was discussed and the need for explaining the occurrence of leaf spot epidemics was indicated. It was shown that epidemics of wildfire (*Bacterium tabacum* Wolf and Foster) are conditional on the occurrence of leaf water soaking, and the factors modifying leaf resistance to water soaking were discussed. These results are extended in the present paper by similar studies with the blackfire (*Bact. angulatum* Fromme and Murray) disease.

Blackfire is the common tobacco leaf spot disease of Virginia, North Carolina, Tennessee, Kentucky, and Wisconsin. Following its identification by Fromme and Murray,[3] it was long regarded as established that the disease was caused by *Bacterium angulatum* and was serious and destructive. It was a fact, however, that no one had ever produced the "epidemic" type of blackfire (see fig. 2, *A*) under controlled conditions. Inoculations produced only small localized lesions (fig. 1), and in consequence Valleau [4] suggested that the epidemic type of leaf spot was nonparasitic in nature. It is believed that the results presented in this paper adequately clear up this situation.

EXPERIMENTAL RESULTS

Methods of study and preliminary results with *Bacterium angulatum* paralleled in all details those given in a previous publication [5] for *Bact. tabacum*.

In repeated controlled experiments, it was demonstrated that the difference between disease development as shown in figure 1 and figure 2, *A*, was entirely dependent on leaf water soaking (fig. 3). Thus, for example, 12 leaves were selected and one-half of each was sprayed until a water-soaked condition of these areas was produced. Sixty-five of the water-soaked areas were then inoculated by pricking them lightly with a needle dipped in a suspension of the bacteria, and an equal number of inoculations were made on the unsprayed halves. The remaining water-soaked areas were left uninoculated. The inoculations of water-soaked areas produced lesions in every case, ranging

[1] Received for publication Aug. 18, 1937; issued February 1938.

[2] CLAYTON, E. E. WATER-SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE WILDFIRE DISEASE OF TOBACCO. Jour. Agr. Research 52: 239–269, illus. 1936.

[3] FROMME, F. D., and MURRAY, T. J. ANGULAR-LEAF SPOT OF TOBACCO, AN UNDESCRIBED BACTERIAL DISEASE. Jour. Agr. Research 16: 219–228, illus. 1919.

[4] VALLEAU, W. D. ARE BLACKFIRE AND ANGULAR LEAF SPOT OF TOBACCO IDENTICAL? (Abstract) Phytopathology 19: 93. 1929.

[5] CLAYTON, E. E. See footnote 2.

from ⅜ to 1⅞ inches in diameter. The inoculations without water soaking produced either no infection or at most a lesion less than one-eighth of an inch in diameter. The water-soaked condition of areas that were not inoculated disappeared without any leaf injury. Furthermore, the lesions on the water-soaked areas developed so rapidly that they were of large size when the plants were removed from the damp chambers (60 hours after inoculation).

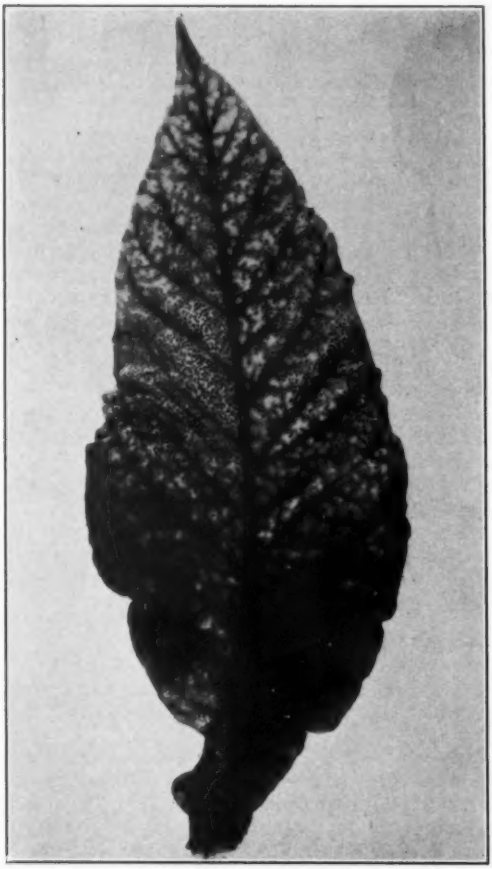

FIGURE 1.—Leaf inoculated by atomizing with a *Bacterium angulatum* suspension; photographed 20 days later. The lesions have attained full size and are typical of those obtained without the aid of water soaking.

INOCULATION OF UPPER AND LOWER LEAF SURFACE

In the work with wildfire it was found that, provided water soaking was present, there was little or no difference in the results from lower or upper leaf-surface inoculation. With *Bacterium angulatum*, which appears to be a less virulent parasite, there were significant differences in results between the two surfaces (table 1).

TABLE 1.—*Effects of different combinations of water-spray and inoculation treatments on the upper and lower leaf surfaces*

Leaf no.	Leaf surface given—		Initial lesions	Leaf area finally killed	Leaf no.	Leaf surface given—		Initial lesions	Leaf area finally killed
	Water spray	Inoculation				Water spray	Inoculation		
			Number	*Percent*				*Number*	*Percent*
1	Upper	Upper	36	20	1	Lower	Upper	125	20
2	do	do	36	50	2	do	do	13	10
3	do	do	20	20	3	do	do	70	10
1	do	Lower	250	80	1	do	Lower	224	40
2	do	do	154	50	2	do	do	168	20
3	do	do	150	100	3	do	do	450	50

Thus, with *Bacterium angulatum*, even with water soaking, the number of initial lesions and usually the subsequent disease damage were much greater from inoculation of lower leaf surfaces, though it did not appear to matter greatly whether the water soaking was produced by spraying upper or lower surfaces. The check inoculations of leaves not soaked that accompanied this experiment produced only scattering small lesions, and these caused practically no leaf damage.

EFFECT OF SIZE AND PERSISTENCE OF WATER-SOAKED AREAS ON LESION DEVELOPMENT

Experiments with size of water-soaked areas in relation to blackfire development showed that, as with wildfire, larger water-soaked areas

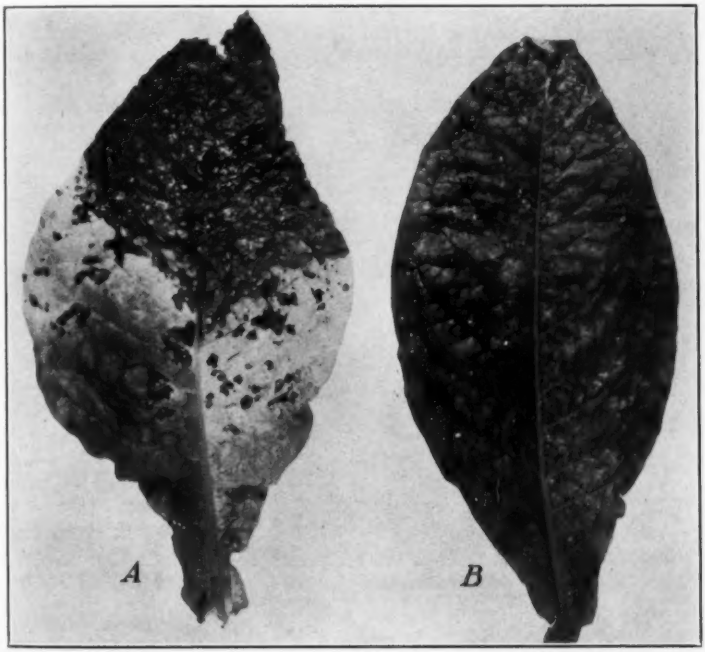

FIGURE 2.—The blackfire disease as it developed from artificial inoculation under favorable field conditions: *A*, Lower leaf, showing typical symptoms of the destructive epidemic type of disease which is usually called blackfire; *B*, upper leaf from the same plant, showing lesions of a type frequently described as angular leaf spot.

favored the development of larger lesions. Thus, the following results were obtained:

Leaves not water-soaked.—Twenty prick inoculations gave either no infection or a mere trace.

Leaves water-soaked, areas one-eighth to three-eights of an inch in diameter.—20 prick inoculations gave lesions ranging from one-eighth to three-eighths of an inch in diameter.

Leaves water-soaked, areas ¾ to 1½ inches in diameter.—Lesions ranged from one-fourth to three-fourths of an inch in diameter.

With small areas, the average size of the lesions was 0.3 inch, which was about the average size of the original water-soaked areas. The

lesions with largew ater-soaked areas averaged 0.7 inch in diameter, but this was much less than the size of the original water-soaked areas.

FIGURE 3.—Entire leaf inoculated with a *Bacterium angulatum* suspension. Right half previously water-sprayed; left half not water-sprayed.

Repeated tests have shown that this is the usual condition, namely, that small water-soaked areas are completely invaded but larger areas are not.

The time that the water-soaked leaf condition persists after infection has occurred is of great importance in blackfire development, as is shown in table 2 and figures 4 and 5.

The actual number of infections secured was about the same when the leaves were water-soaked for 10, 24, or 50 hours, but with the shorter periods development of the lesions was cut short and damage to the plant was slight. Figure 4, *A*, shows that it is quite possible for leaves to be freely infected by *Bacterium angulatum* and then, even after epidemic disease development is well under way, the progress of the disease can be abruptly checked. These results explain why even severe storms, when followed at once by clear weather, are not effective in producing blackfire outbreaks.

TABLE 2.—*Relation of persistence of water-soaked areas to disease development*

Leaf no.	Leaf area killed after 10 days when water soaking was continued for—			Leaf no.	Leaf area killed after 10 days when water soaking was continued for—		
	10 hours	24 hours	50 hours		10 hours	24 hours	50 hours
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	1	3	20	8	2	3	20
2	1	8	15	9	1	5	75
3	1	5	15	10	2	10	60
4	1	5	25	11	2	8	75
5	2	3	25	12	2	8	75
6	1	5	25				
7	6	2	15	Average	1.8	5.4	37.1

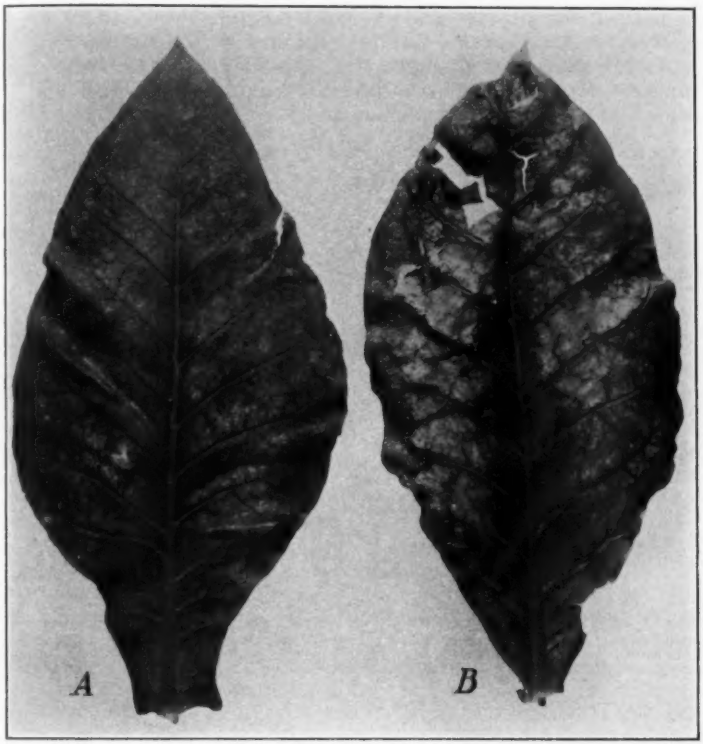

FIGURE 4.—Effect of duration of water-soaked condition on disease development. Both leaves were water-soaked and inoculated alike. With *A* the water-soaked condition was maintained in a saturated atmosphere for 24 hours; with *B*, for 50 hours. Note the heavy infection but arrested disease development on *A;* with *B*, the final result was extensive tissue destruction.

EFFECT OF TOPPING AND FERTILIZATION PRACTICES ON SUSCEPTIBILITY TO BLACKFIRE

Height of topping has a marked effect on the susceptibility of the host plant to epidemic blackfire, just as was previously shown for wildfire. In table 3 results are given from a field test conducted in 1935. All plots were uniformly inoculated and conditions were moderately favorable for disease development. Low-topped plants averaged 10 leaves per plant; high-topped plants, 18 leaves; plants not topped, 25 leaves. The percentage of leaf area destroyed by disease was estimated for 40 leaves from each plot.

Topping effects are at once marked and consistent, and low topping is undoubtedly a major factor in promoting blackfire damage in the dark-fired tobacco-producing areas where this practice is essential to produce a large, heavy type of leaf.

It was not possible to make so detailed a study of fertilizer influences on blackfire development as was made on wildfire development. However, numerous observations indicate clearly that low-potash and high-nitrogen fertilization increase blackfire damage. These fertilizer effects were very apparent in plot work conducted on soils of

low natural fertility, where the same fertilization treatments were repeated year after year; but on moderately fertile soils, with the application made but a single year, even marked variations in the quantities of nitrogen and potash applied in the fertilizer had but little effect on blackfire development.

FIGURE 5.—Effect of water soaking and duration of water-soaked condition on blackfire development for entire plants. Both plants were water-soaked and inoculated alike. *A* was then held in a saturated atmosphere for 10 hours; *B*, for 50 hours. Photographed 12 days later.

TABLE 3.—*Percentages of leaf area killed following different heights of topping*

Low topping[1]	High topping[1]	No topping[1]
60–30– 8–20–15	2– 5–30– 5–30	8– 2– 5– 2– 2
5–12–20–15– 5	10–10–10– 6–10	5– 1– 5– 1– 4
2– 4– 6–10–20	2– 5– 3–15– 5	4– 1– 2– 2– 3
10–12– 4–50–10	10– 5– 4– 8– 6	.5–. 5– 2–. 5– 2
30–10–20– 8–30	8– 3–10–10–10	3– 5– 2– 8–25
25–15– 5–10– 2	5– 8– 8– 2– 8	4– 5–20–40– 1
20–12– 5–35–30	8–40– 2– 8– 8	2– 5– 3– 4– 5
20–25–20–30– 2	5–30– 8– 4–15	.5– 1– 2– 2–25
Average 16. 8	Average 9. 5	Average 5. 4

[1] Each of the following 40 percentages refers to a separate leaf. The values are arranged in groups of 5 merely for convenience and to save space. No relationship exists among values occupying similar positions in different columns.

DISCUSSION

The results secured in these experiments with blackfire, caused by *Bacterium angulatum*, are similar in every respect to those previously reported for wildfire, and it seems clear that with types of tobacco grown in the United States epidemic development of blackfire is dependent on water soaking of the leaves. Water soaking breaks down host resistance and permits successful and rapid tissue invasion by the organism.

Bacterium angulatum, while similar in mode of action to *Bact. tabacum*, is evidently a less virulent parasite. Thus, throughout the work

in parallel experiments, infection with *Bact. angulatum* in the absence of water soaking was less easy to secure and the infections were less numerous, developed more slowly, and were smaller; with water soaking and more favorable conditions, these differences still persisted though they were not so marked. With favorable conditions, both organisms can practically destroy a crop in a short time.

Some degree of blackfire protection can be secured by high topping, and low-nitrogen and high-potash fertilization is also helpful, but these practices can be applied only in areas where the type of tobacco grown will permit. They are applicable to the flue-cured area.

However, it is evident that effective blackfire control must be sought by other means, and the most promising of these are (1) sanitation and other measures designed to eliminate sources of infection and (2) development of varieties still more resistant to the disease than those now available. In the latter connection it is to be noted that varieties grown in the United States are practically all moderately resistant to blackfire, which accounts for the fact that they suffer little damage from the disease until this resistance is broken down by the conditions incident to heavy storms.

SUMMARY

Tobacco leaves are readily infected by *Bacterium angulatum*, but under ordinary conditions invasion is limited to small areas. The lesions are usually one-eighth of an inch or less in diameter, and large numbers of infections cause but little damage to most types of tobacco.

It had been suggested that the large, quickly developing lesions characteristic of epidemic blackfire were nonparasitic in nature; but it is now shown that they are caused by *Bact. angulatum*, but only under special conditions. The resistance of the leaf to invasion must first be broken down by water soaking, which in turn results from severe storms.

Even after leaves are water soaked, however, and infection has occurred, the development of the disease is abruptly checked if the water-soaked condition disappears within a few hours. Epidemic disease development was repeatedly obtained by water soaking the leaves for 48 hours. Resistance of the leaves to water soaking, and hence to the disease, has been shown to be greatly modified by topping and fertilization practices. High topping and low-nitrogen and high-potash fertilization increase leaf resistance to water soaking, and these measures are recommended as practicable in the flue-cured tobacco area.

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ROLE OF MOLYBDENUM IN THE UTILIZATION OF AMMONIUM AND NITRATE NITROGEN BY ASPERGILLUS NIGER [1]

By ROBERT A. STEINBERG

Associate physiologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

A study of the factors associated with the very poor growth and sporulation of *Aspergillus niger* Van Tiegh under certain conditions of molybdenum deficiency, briefly mentioned in a former publication (*16*),[2] is reported in this paper. The evidence obtained renders it quite probable that one of the functions of molybdenum in plants is in the reduction processes whereby ammonium nitrogen is formed from nitrate nitrogen; that is, the marked effects of molybdenum deficiency appear to be associated with the presence and utilization of nitrate nitrogen. Its action seems to be unique in this regard, inasmuch as acidity, iron, zinc, copper, and manganese do not act similarly.

The data of prior investigations on the utilization of ammonium and nitrate nitrogen by green plants (*1*, *2*, *10*, *11*) and by fungi (*12*, *13*) have been explained variously on the basis of hydrogen-ion concentration and of iron, copper, and manganese concentration. The consensus of opinion is that the processes of nitrate utilization are quite similar in green plants and fungi (*4*) and culminate in the formation of ammonium ion for the synthesis of amino acid and protein. Certain differences exist, nevertheless. Green plants would appear to thrive best with nitrate nitrogen as a rule, though ammonium nitrogen can give results fully as good if the environmental factors are properly adjusted. Fungi, however, would appear to be more tolerant of variations in concentration of ammonium ion and other components of the nutrient solution. Moreover, certain genera of fungi have been found unable to utilize nitrate nitrogen (*7*, *9*). Experimental emphasis in the data on nitrogen utilization here reported has been placed on the maximum yield obtainable at a fixed level of 665 mg of nitrogen per liter. This is a minimum value and corresponds to that employed in the optimum solution (dibasic) containing 1.90 g of ammonium nitrate (NH_4NO_3) per liter (*16*). This procedure is based on the assumption that equal growth with two different forms of nitrogen under conditions equal or optimum in all other respects implies that the rate and extent of utilization of each are identical and therefore that no intrinsic difference in availability exists.

METHODS

The organism used in these experiments was *Aspergillus niger* ("W" strain). It was grown at 35° C. in 50-cc portions of a 5-percent sugar solution containing all necessary salts. Water redistilled in

[1] Received for publication July 2, 1937; issued February 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 901.

pyrex glassware, sucrose containing not more than 0.0025 percent of ash, and reagent chemicals were used throughout. The mycelial felts were harvested, dried at 103°, and weighed as usual. Acidities were determined with a quinhydrone electrode. Earlier papers by the writer (*14*, *15*, *16*) should be consulted for additional details.

NUTRIENT VALUE OF VARIOUS FORMS OF NITROGEN

The experimental data of tables 1 to 3, inclusive, form a series designed to test four different nitrogen sources as regards their suitability for growth and their effect on the trace-element requirements of *Aspergillus niger*. The solution used in the experiments of table 1 is the dibasic optimum solution (*16*), except that sodium nitrate was tested also with a solution in which monopotassium phosphate was substituted for the dipotassium phosphate of the optimum solution. Equivalent quantities of ammonium nitrate, ammonium chloride, sodium nitrate, and urea, at a nitrogen level of 665 mg per liter, were employed. The experiments of table 2 are a repetition of those of table 1, except that each trace element (iron, zinc, copper, manganese, and molybdenum) has been increased where necessary to the minimum concentration that is optimum for growth. These experiments are again duplicated in table 3, but this time each trace element is present in minimum optimum quantity and the potassium phosphate and magnesium sulphate have each been increased 40 percent.

TABLE 1.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 5 days, when heavy-metal concentrations optimum for NH_4NO_3 were employed*

Element omitted	NH_4NO_3				NH_4Cl				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	236.1	21.14	1.90	5,bl	502.6	49.90	1.59	3,j	214.8	31.62	2.47	4,j
Zn	52.5	4.68	2.60	4,bl	90.5	9.39	2.27	4,j	42.6	6.27	2.69	2,j
Cu	991.7	88.48	2.81	2,y	918.3	91.16	1.38	1,w	616.9	90.82	2.51	4,t
Mn	766.0	68.38	1.67	2,bl	726.3	72.11	1.37	2,j	737.8	108.92	2.17	4,bl
Mo	800.4	71.42	1.73	3,bl	1,025.6	101.82	1.36	2,j	724.0	106.59	2.24	8,bl
None [5]	1,120.8	100.00	2.64	6,bl	1,007.3	100.00	1.35	2,j	679.2	100.00	2.33	10,bl
Maximum	[6] 1,131.5				[6] 1,070.0				[6] 832.4			
C. U		[7] 45.26				[7] 42.80				[7] 33.30		
pH			[8] 7.14				[8] 7.01				[8] 6.98	

See footnotes at end of table.

TABLE 1.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 5 days, when heavy-metal concentrations optimum for NH_4NO_3 were employed*—Continued

Element omitted	$NaNO_3$				$NaNO_3$ [2]			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	144.3	20.37	3.28	4,j	282.2	29.10	3.20	8,bl
Zn	31.6	4.45	3.06	2,j	61.3	6.32	2.99	3,j
Cu	698.0	98.53	2.75	2,t	1,013.8	104.53	2.96	6,t
Mn	655.6	92.54	1.89	2,j	937.2	96.63	2.05	1,j
Mo	73.4	10.35	3.03	3,j	83.0	8.55	2.69	3,j
None [3]	708.5	100.00	2.99	8,bl	969.9	100.00	2.97	10,bl
Maximum	[6] 748.5				[6] 1,035.4			
C. U		[7] 29.94				[7] 41.42		
pH			[8] 7.45				[8] 4.91	

[1] Water, 1,000 cc; sucrose, 50 g; NH_4NO_3, 1.90 g; K_2HPO_4, 0.35 g; and $MgSO_4.7H_2O$, 0.25 g.
[2] Used 0.55 g of KH_2PO_4 instead of 0.35 g of K_2HPO_4.
[3] Yield from complete medium, with none of the trace elements omitted, taken as 100 percent.
[4] Spore color is indicated by the initial letter or letters of the terms "jet," "black," "brown," "tan," "yellow," or "white," and extent of sporulation by the numerals 0 (no spores) to 10 (entirely covered with spores).
[5] Trace-element concentrations (milligrams per liter) were as follows: Fe, 0.20; Zn, 0.18; Cu, 0.04; Mn. 0.02; and Mo, 0.02.
[6] Maximum individual yield.
[7] Coefficient of utilization, or yield per 100 g of sucrose.
[8] Initial pH of nutrient solution.

TABLE 2.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentrations were employed*

Element omitted	NH_4NO_3				NH_4Cl				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [2]	Acidity at harvest	Sporulation [4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	234.3	22.45	2.00	3, bl	466.6	48.03	1.67	4, bl	181.6	20.15	2.27	2, bl
Zn	118.6	11.36	2.41	4, bl	43.2	4.45	1.93	4, bl	100.2	11.11	2.71	2, bl
Cu	965.6	92.51	2.38	1, w	915.3	94.22	1.44	0	821.4	91.10	2.68	4, t
Mn	742.0	71.09	1.58	1, w	749.4	77.14	1.35	1, bl	784.6	87.03	1.96	4, bl
Mo	690.5	66.16	1.65	2, bl	974.3	100.30	1.42	3, bl	908.8	100.80	2.10	10, bl
None [5]	1,043.8	100.0	1.96	8, bl	971.4	100.00	1.46	6, bl	901.6	100.00	1.99	10, bl
Maximum	[6] 1,059.4				[6] 975.8				[6] 1,002.3			
C. U		[7] 42.38				[7] 39.03				[7] 40.09		
pH			[8] 7.29				[8] 7.13				[8] 7.37	

See footnotes at end of table.

TABLE 2.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution*[1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentrations were employed*—Continued

Element omitted	$NaNO_3$				$NaNO_2$[2]			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	199.8	20.67	3.25	8, bl	240.3	25.05	3.36	6, bl
Zn	203.4	21.05	3.16	8, bl	280.6	29.25	2.97	6, bl
Cu	861.4	89.13	3.86	6, t	963.2	100.39	4.17	6, t
Mn	889.5	92.03	2.11	6, bl	824.6	85.93	2.05	0
Mo	104.7	10.84	3.30	6, bl	159.8	16.76	3.27	8, bl
None[5]	996.5	100.00	2.81	10, bl	959.5	100.00	3.19	10, bl
Maximum	[6] 983.6				[6] 965.6			
C. U.		[7] 39.44				[7] 38.62		
pH			[8] 7.45				[8] 4.85	

[1] See footnote 1, table 1.
[2] See footnote 2, table 1.
[3] See footnote 3, table 1.
[4] See footnote 4, table 1.
[5] Trace-element concentrations (mg per liter) were as follows: With NH_4NO_3: Fe, 0.24; Zn, 0.26; Cu, 0.04; Mn, 0.03, and Mo, 0.03. With NH_4Cl: Fe, 0.20; Zn, 0.20; Cu, 0.05; Mn, 0.02; and Mo, 0.02. With urea: Fe, 0.24; Zn, 0.26; Cu, 0.04; Mn, 0.03; and Mo, 0.04. With $NaNO_3$: Fe, 0.24; Zn, 0.30; Cu, 0.08; Mn, 0.05; and Mo, 0.06. With $NaNO_3$ (acid): Fe, 0.20; Zn, 0.22; Cu, 0.04; Mn, 0.02; and Mo, 0.02.
[6] See footnote 6, table 1.
[7] See footnote 7, table 1.
[8] See footnote 8, table 1.

TABLE 3.—*Effects of different nitrogen compounds (665 mg of N per liter), in an optimum solution*[1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentration and a 40-percent excess of potassium phosphate and magnesium sulphate were employed.*

Element omitted	NH_4NO_3				NH_4Cl				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained[2]	Acidity at harvest	Sporulation[4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	276.9	26.31	1.93	4, bl	363.7	35.36	1.79	6, bl	243.8	26.69	2.54	2, bl
Zn	136.0	12.83	2.38	5, bl	34.8	3.39	2.63	4, bl	36.5	4.00	2.84	2, bl
Cu	886.0	84.19	2.82	2, w	970.7	94.38	1.42	1, w	909.5	98.46	2.48	2, br
Mn	870.0	82.66	1.65	2, bl	795.0	77.30	1.41	2, bl	822.1	89.98	2.27	2, bl
Mo	680.6	64.67	1.83	2, bl	1,017.6	98.93	1.42	2, bl	1,008.1	110.34	2.49	8, bl
None[5]	1,052.4	100.00	2.15	8, bl	1,028.5	100.00	1.44	4, j	913.6	100.00	2.26	8, bl
Maximum	[6] 1,070.1				[6] 1,036.0				[6] 1,142.7			
C. U.		[7] 42.80				[7] 41.44				[7] 45.71		
pH			[8] 7.36				[8] 7.23				[8] 7.68	

See footnotes at end of table.

TABLE 3.—*Effects of different nitrogen compounds (665 mg of N per liter), in an optimum solution*[1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentration and a 40-percent excess of potassium phosphate and magnesium sulphate were employed*—Continued

Element omitted	$NaNO_3$				$NaNO_3$[2]			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	259.5	24.60	3.27	4, t	266.9	26.87	3.32	4, bl
Zn	60.6	5.95	3.08	4, bl	119.7	12.06	2.98	2, bl
Cu	825.5	83.72	4.12	2, t	929.0	93.54	4.60	3, t
Mn	993.6	97.58	2.27	3, bl	865.1	87.10	2.04	0
Mo	81.0	7.95	3.05	3, bl	118.9	11.97	3.00	4, bl
None[3]	1,018.3	100.00	2.94	10, bl	994.9	100.00	3.33	10, bl
Maximum	[6] 1,035.5				[6] 1,005.6			
C. U		[7] 41.42				[7] 40.22		
pH			[8] 7.46				[8] 4.93	

[1] See footnote 1, table 1.
[2] See footnote 2, table 1.
[3] See footnote 3, table 1.
[4] See footnote 4, table 1.
[5] See footnote 5, table 2.
[6] See footnote 6, table 1.
[7] See footnote 7, table 1.
[8] See footnote 8, table 1.

The data of table 1 may be summarized by the statement that compared to the yield obtained with the dibasic optimum (NH_4NO_3) the maximum yields were low only with urea and sodium nitrate (pH 7.45). Deficiency tests with the trace elements in these solutions yielded poorer results on the whole than with ammonium nitrate, but only in the case of molybdenum deficiency in both the alkaline and acid sodium nitrate solutions did a significant difference appear. The effects of molybdenum deficiency in the latter solutions were a very marked diminution in yield and sporulation. The cultures were typical in appearance of those suffering from a marked nutrient deficiency of such elements as iron, zinc, sulphur, nitrogen, magnesium, or phosphorus (*14*).

In table 2, with optimum concentrations of trace elements, as well as in table 3, where trace elements were in optimum concentration and potassium phosphate and magnesium sulphate were in moderate excess, the maximum yields with all solutions showed good agreement. The effects of trace-element deficiencies were quite similar to those in the experiments of table 1. Examination of these tables reveals that the molybdenum deficiency tests with urea and ammonium salt gave the poorest results, those with ammonium and nitrate (NH_4NO_3) gave results of intermediate value, and those with nitrate gave the most striking results. Neither moderate increase in acidity and trace elements nor increased quantities of the other essential elements modified these results appreciably.

Though for reasons that will be stated later it seemed quite certain that the difference in results with ammonium nitrogen and nitrate nitrogen were associated with a higher molybdenum requirement in nitrate solutions, these experiments were extended to include other salts containing ammonium or nitrate nitrogen. Tests were also made of the effects at a marked acidity, and of nitrogen supplied as asparagine. These results have been tabulated in tables 4 and 5.

TABLE 4.—*Miscellaneous experiments on the effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days*

Element omitted	$NaNO_3$ [2] (high acidity)				$Ca(NO_3)_2.4H_2O$				KNO_3			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	319.9	34.98	3.12	6, b1	139.6	14.03	3.12	4, b1	235.5	22.88	3.26	6, b1
Zn	93.1	10.18	3.16	6, b1	121.0	12.17	2.99	4, b1	100.9	9.80	3.04	4, b1
Cu	947.1	103.56	3.23	4, t	879.0	88.40	3.28	2, y	1,047.7	101.77	3.87	8, t
Mn	526.7	57.59	2.07	0	539.2	54.22	3.14	0	859.3	85.48	2.11	0
Mo	245.7	26.86	3.02	6, b1	880.9	88.59	3.34	8, br	150.5	14.62	3.05	8, br
None [5]	914.5	100.00	3.05	9, b1	994.4	100.00	3.57	10, br	1,029.4	100.00	3.08	10, br
Maximum	[6] 1,067.4				[6] 1,021.8				[6] 1,069.8			
C. U		[7] 42.70				[7] 40.87				[7] 42.79		
pH			[8] 2.48				[8] 4.63				[8] 4.93	

Element omitted	$(NH_4)_2SO_4$				$Ca(NO_3)_2.4H_2O$			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	254.8	24.92	2.12	4, b1	66.4	6.59	3.06	2, b1
Zn	71.9	7.03	2.56	4, b1	99.0	9.82	2.98	2, b1
Cu	926.0	90.55	1.54	0	914.7	90.87	2.36	6, t
Mn	629.0	61.21	1.54	0	797.5	79.23	1.66	0
Mo	1,018.6	99.61	1.51	1, b1	938.0	93.19	2.72	8, b1
None [5]	1,022.7	100.00	1.52	2, b1	1,006.6	100.00	2.73	8, b1
Maximum	[6] 1,046.3				[6] 1,207.8			
C. U		[7] 41.85				[7] 48.31		
pH			[8] 5.08				[8] 4.47	

[1] See footnote 1, table 1.
[2] See footnote 2, table 1.
[3] See footnote 3, table 1.
[4] See footnote 4, table 1.
[5] See footnote 5, table 1.
[6] See footnote 6, table 1.
[7] See footnote 7, table 1.
[8] See footnote 8, table 1.

TABLE 5.—*Additional miscellaneous experiments on the effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days*

Element omitted	NH_4Cl+NaCl (0.2 percent)				Asparagine				$LiNO_3$			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [2]	Acidity at harvest	Sporulation [3]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	386.0	38.73	1.73	5, bl	260.2	29.97	2.57	10, bl	170.3	22.62	3.33	4, bl
Zn	34.0	3.41	2.68	4, bl	151.6	17.47	2.61	10, bl	96.8	12.86	3.10	4, bl
Cu	936.0	93.94	1.42	2, w	1,110.9	127.96	2.52	4, t	803.6	106.73	3.93	2, w
Mn	730.8	73.34	1.39	2, bl	1,088.3	125.35	2.23	4, bl	784.9	104.25	2.09	0
Mo	979.5	98.30	1.40	3, bl	940.9	108.38	2.40	10, bl	110.8	14.71	3.09	6, bl
None [4]	996.5	100.00	1.40	6, bl	868.2	100.00	2.18	10, bl	752.9	100.00	3.62	8, bl
Maximum	[5] 1,051.5				[5] 1,136.6				[5] 997.6			
C. U		[6] 42.06				[6] 45.46				[6] 39.90		
pH			[7] 7.02				[7] 6.56				[7] 4.78	

Element omitted	$Mg(NO_3)_2.6H_2O$				NH_4NO_3 (purified with $CaCO_3$)			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	275.4	29.18	3.02	6, bl	18.8	1.59	2.85	2, bl
Zn	157.2	16.66	2.91	6, bl	12.5	1.05	2.94	2, bi
Cu	1,017.3	107.78	2.74	6, t	917.1	77.61	2.51	2, w
Mn	1,046.7	110.90	2.08	2, bl	1,056.3	89.40	1.67	2, bl
Mo	152.4	16.15	2.64	4, bl	1,067.7	90.36	2.15	8, bl
None [4]	943.8	100.00	2.50	10, bl	1,181.6	100.00	2.49	10, bl
Maximum	[5] 1,051.9				[5] 1,215.7			
C. U		[6] 42.08				[6] 48.63		
pH			[7] 4.51				[7] 6.94	

[1] See footnote 1, table 1.
[2] See footnote 3, table 1.
[3] See footnote 4, table 1.
[4] See footnote 5, table 1.
[5] See footnote 6, table 1.
[6] See footnote 7, table 1.
[7] See footnote 8, table 1.

A study of all the tables reveals that, except in the case of calcium nitrate, the omission of molybdenum resulted in the greatest loss in yield and sporulation with nitrate nitrogen and the least loss with ammonium nitrogen and organic nitrogen (urea, asparagine). Neither high acidity nor the possible influence of the cation introduced with the nitrate nitrogen seems to have been a factor. The extent of growth with deficiency of the trace elements other than molybdenum varied to about the same degree as in previous experiments, though it seemed on the whole to be somewhat larger than with ammonium nitrate. Though the solution with magnesium nitrate gave results that were quite poor with iron, zinc, manganese, and copper deficiencies, the results with molybdenum deficiency were still excellent.

Comparison of the deficiency yields with sodium nitrate at the three different levels of acidity brings additional confirmation of previous statements by the writer (*15*) that acidity decreases the optima or

increases the availability of the trace elements iron, zinc, copper, and manganese. Molybdenum, it is now noted, behaves similarly; it is also noted that the beneficial influence of acidity is limited at the highest pH value in the case of manganese. The combined deleterious effect of excess acidity and manganese deficiency on yield and sporulation becomes evident at even moderate acidities.

DISCUSSION

A comparison of the maximum yields obtainable with ammonium nitrogen and nitrate nitrogen in these experiments would indicate full nutritive equivalence of these forms of nitrogen. The maximum individual yield with ammonium nitrogen was 1,051.8 mg and with nitrate nitrogen 1,078.9 mg, the difference amounting to only 2.58 percent of the smaller number. The data, moreover, afford an explanation of previous claims to the contrary. Experimental evidence appearing to support this latter view can be readily obtained if the precaution of adjusting each of the other components for maximum efficiency is overlooked. However, tables 1 to 3 present unquestionable evidence that it is experimentally unsound to omit a check on each of the other known components of a nutrient solution before ascribing observed effects wholly to variations in even the better known constituents. Many of the effects attributed in the literature to variation in composition or concentration of nutrient salts of nitrogen, potassium, etc., are almost certainly due partly to chance variations in the essential trace elements which the salts contain as impurities.

No verification could be obtained of the claims for special effects of acidity, iron, copper, or manganese on the utilization of nitrates. Their effects on this process are quite similar to those of other nutrients. The action of increased acidity, as already pointed out, is to make more available the trace elements present so that growth can proceed further. This decrease in the optimum concentration of the trace elements might readily be attributed to the direct action of increased acidity though primarily due to the elements themselves. The sharply contrasting results with molybdenum in ammonium and nitrate solutions are unique and not shared by the other heavy metals studied.

The results with molybdenum might conceivably be due either to the relative freedom of nitrates from this element or to a greater requirement in the presence of nitrates. Chemical determination of the molybdenum content of the reagents would be the logical and definite method of deciding this question, but lack of time and facilities rendered this procedure impracticable. Other evidence nevertheless affords sufficient aid to enable one to make a decision.

The assumption of a greater molybdenum content in the ammonium salts supplying nitrogen is not substantiated by the impurity content of the other trace elements in these salts, since when they were employed in deficiency tests the percentages of maximum yield were approximately the same. Neither do the solutions employing nitrate nitrogen all require the addition of greater quantities of molybdenum to bring about maximum growth and sporulation. Lastly, spectroscopic examination failed to reveal the presence of molybdenum in the ammonium nitrate used, though a trace was found in the sucrose (*16*).

Similar results, too, were obtained with a sample of ammonium sulphate of spectroscopic purity. All the evidence available supports the assumption that nitrates act through their presence and not because of their relative freedom from molybdenum impurity; that is to say, the need of the fungus for molybdenum is greater when nitrogen is supplied as nitrate. Or, phrased differently, the trace of molybdenum unavoidably present in the nutrient solution suffices for a greater production of mass with ammonium nitrogen than with nitrate nitrogen.

It should not be assumed, however, that it is solely under such conditions that molybdenum is required. The omission of molybdenum from the nutrient solution causes a slight but definite diminution in yield with other sources of nitrogen than nitrate. With improved purity of reagents a still greater diminution in yield will probably be obtained with ammonium salts also when molybdenum is withheld. Though these results lead naturally to the assumption that molybdenum functions in the reduction processes of the fungus whereby nitrate is reduced to ammonia, molybdenum doubtless functions in other metabolic processes as well. If this viewpoint be correct, the possibility further suggests itself that the macroscopic appearance of the fungus when molybdenum is withheld may be due primarily to nitrogen starvation even though nitrate is present. The symptoms of molybdenum deficiency with nitrate nitrogen are quite similar to those of nitrogen deficiency, but this is true also of all the others except potassium, manganese, and perhaps copper. The symptoms of molybdenum deficiency may be found, therefore, to be somewhat different with ammonium nitrogen.

Interpolation here of some observations on the response of the fungus to molybdenum deficiency when nitrogen is supplied as calcium nitrate will not be amiss in view of the very frequent use of this salt in nutrient solutions for green plants. Both of the samples of this salt that were tested would appear to be contaminated with molybdenum to such an extent as to render them unfit for deficiency studies on this element. This condition is believed not to be accidental but to be due to the comparative difficulty of obtaining this salt in a sufficient degree of purity.[3] Lithium, sodium, potassium, magnesium, and even ammonium nitrate, of reagent grade, appear much more suitable for studies of molybdenum deficiencies. This variation in percentage of molybdenum impurity of different samples of nitrates may be a partial explanation, moreover, of their reported varying effectiveness in nutrient solution and as fertilizer.

The importance of the impurities present, even in the purest reagents now commercially available for use in studying the responses of plants to nutrient variations in culture, is greatly underestimated by many investigators. The many elements that may thus be accidentally included are listed in the tabulation that follows. Even this tabulation is inadequate, however, since the spectroscope is unsatisfactory foidentification of some of the chemical elements at concentrations bior logically significant. Tentatively, it is estimated that if an element is present in quantities greater than 1 part per billion one cannot be

[3] This opinion is fully substantiated by data obtained shortly after this manuscript was accepted for publication. Extreme differences between the molybdenum content of reagent calcium nitrate of different manufacturers is indicated; some samples contain traces of molybdenum ample for the needs of the organism, whereas others do not. Data not yet published show that marked decreases in yield occurred in certain instances with calcium nitrate and also with strontium nitrate and with barium nitrate when molybdenum in the nutrient solution was deficient in quantity.

reasonably certain that it is unessential. This degree of purity is seldom attainable at present. The only safeguards available are a thorough check of all components of the specific culture solution used and their employment in the minimum quantities sufficing for maximum growth under the conditions employed. Neglect of these precautions is undoubtedly the cause of many of the conflicting findings reported in the literature.

Impurities identified spectroscopically as present in some of the reagents[1] *used in nutrient solutions for Aspergillus niger*[2]

Compound:	*Contaminants identified spectroscopically*[3]
NH_4NO_3	Na, Mg, Ca, K(?).
K_2HPO_4	Al*, Pb*, Na*, Ca*, Mg, Ag.
$MgSO_4.7H_2O$	Na, Cu.
$FeSO_4.7H_2O$	
$ZnSO_4.7H_2O$	Fe, B(?), As, Mg*, Sn(?), Cu, Si, Na, Mn.
$CuSO_4.5H_2O$	Fe, Mn, Si, Mg, Ca, Pb.
$MnSO_4.2H_2O$	Na, Fe, Cu, Al, V, Cr, Si*, Mg*, Ca*.
Na_2MoO_4	Cu, Mn, Fe, Al, Ni, Mg, Ca*, K, Na, Mn, Si, Li, V(?).
Dextrose[4]	Li, Na, Sr, Ca, Rb, K, Mn, Al, Fe, Rh, Ni, Ag, Cu, Mg, Sn, B, Si.
$SrCl_2.6H_2O$	Na, Mg, Ba. Ru, Ti(?), Pt, Ir, Ca*, Mn, Pd, Si, Tl(?).

[1] These data were obtained by B. C. Brunstetter, associate biochemist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, with a Bausch & Lomb large-size quarts spectrograph and carbon arc. No attempt was made to achieve prior chemical concentration or separation of the impurities, however.

[2] The following elements were found in the ash of *Aspergillus niger* felts grown in a dibasic optimum solution with the above chemicals: K, Mg, Fe, Cu, Mn, Na, Ca, Sr, Ba, Al, Pb, Ag. Ti(?), V(?).

[3] Strong traces are indicated by asterisks (*), doubtful by question marks.

[4] This compound was not employed but is inserted for the purpose of giving an idea of the probable impurities in sucrose. The data are quoted from Lockwood (*8*).

The relation ascertained to exist between nitrates and molybdenum in the metabolism of *Aspergillus niger* also possibly affords a definite chemical basis for explaining certain observations on green plants. Dittrich (*3*) found that extracts of beet tissue gave a negative test for nitrate reductase when the plants were supplied with ammonium nitrogen. This observation has been verified by Tiedjens and Blake (*17*) with extracts of apple tree roots. There would seem, therefore, to be a definite correlation in plants between utilization of nitrate nitrogen, presence of reductase, and high molybdenum requirement as contrasted with utilization of ammonium nitrogen, absence of reductase, and minimum need for molybdenum. It should be remembered in this connection that enzymatic deficiency in consequence of the absence of a suitable substrate has frequently been reported.

The direct determination of the relationship between reductase and molybdenum would be of considerable interest. This element may of course function purely as an inorganic catalyst after the manner reported by Kharasch et al. (*6*) for the oxidation of thioglycolic acid. On the other hand, molybdenum may serve to activate nitrate reductase, because it forms an integral part of the reductase molecule. The activation of urease by certain metal ions in the hydrolytic splitting off of ammonia from arginine is considered necessary for this reason by Kellerman and Perkins (*5*). In both the investigations just cited the metallic ions are required in traces of fractional parts per million only, and are therefore comparable as respects both type of reaction and concentration of metal ions to the requirements of plant metabolism. Moreover, even in vitro, specificity of effect was found

to exist in these reactions. That specificity was not found more complete in vitro is probably due to the limitation of a single reaction, whereas the organism may, and probably does, employ each essential element in several or more reactions simultaneously. Nor, on the other hand, is specificity absolute in the organism, as the phenomenon of nutrient substitution demonstrates.

CONCLUSIONS

Deficiency tests with *Aspergillus niger* in culture solutions indicate that ammonium nitrogen, nitrate nitrogen, and organic nitrogen (urea, asparagine) are equivalent in value for the nutrition of this fungus. It is necessary, however, to adjust correctly the concentration of the other essential components of the nutrient solution, particularly that of iron, zinc, copper, manganese, and molybdenum. Acidity is of minor importance and is effective chiefly through its action in aiding to minimize deficiencies of these trace elements. The response of the organism to molybdenum is unique in that it is definitely associated with the type of nitrogen nutrition. Molybdenum is required to a greater degree by the organism when nitrate is the source of nitrogen than when ammonium or organic nitrogen is the source. Though marked variations in molybdenum content of different lots were found to exist, striking deficiency results were obtained with many of the alkali and alkaline earth nitrates. It is considered, therefore, on the basis of these and of other facts reported in the literature, that molybdenum is essential for activation of nitrate reductase in the reduction processes whereby nitrates are reduced to ammonium for synthesis of amino acid and protein by the plant. It is suggested also that biological specificity is a result of chemical specificity of an element and presumably becomes more complete with increase in the number of reactions in which it simultaneously participates in the metabolism of the organism.

LITERATURE CITED

(1) Arnon, D. I.
1936. influence of hydrogen ion concentration at two seasons on the ammonium and nitrate nitrogen nutrition of barley. (Abstracts of Papers) Amer. Jour. Bot. 23: 695.

(2) ———
1936. effect of season, oxygen supply, copper, and manganese on the ammonium and nitrate nitrogen nutrition of barley. (Abstracts of Papers) Amer. Jour. Bot. 23: 695.

(3) Dittrich, W.
1930. zur physiologie des nitratumsatzes in höheren pflanzen (unter besonderer berücksichtigung der nitratspeicherung). Planta 12: [69]–119, illus.

(4) Itzerott, D.
1936. über die bedingungen der stickstoffaufnahme, vor allem der nitrataufnahme, bei aspergillus niger. Flora [Jena] 131 (n. s. 31): 60–86.

(5) Kellerman, L., and Perkins, M. E.
1935. activation of enzymes. iii. the rôle of metal ions in the activation of arginase. the hydrolysis of arginine induced by certain metal ions with urease. Jour. Biol. Chem. 112: 175–194.

(6) Kharasch, M. S., Legault, R. R., Wilder, A. B., and Gerard, R. W.
1936. metal catalysts in biological oxidations. i. the simple system; thioglycolic acid, buffer, metal, dithiol. Jour. Biol. Chem. 113: 537–555, illus.

(7) LEDEBOER, M. S. J.
1934. PHYSIOLOGISCHE ONDERZOEKINGEN OVER CERASTOSTOMELLA ULMI (SCHWARZ) BUISMAN. 95 pp., illus. Baarn. (Proefschrift . . . van den graad van doctor . . . Rijks–Universiteit te Utrecht).
(8) LOCKWOOD, L. B.
1933. A STUDY OF THE PHYSIOLOGY OF PENICILLIUM JAVANICUM VAN BEIJMA WITH SPECIAL REFERENCE TO THE PRODUCTION OF FAT. Catholic Univ. Amer. Biol. Ser. 13, 35 pp., illus.
(9) ——— WARD, G. E., and MAY, O. E.
1936. THE PHYSIOLOGY OF RHIZOPUS ORYZAE. Jour. Agr. Research 53: 849–857.
(10) MILLER, M. S.
1933. TO THE QUESTION OF THE ABSORPTION OF NITRATE, NITRITE AND AMMONIA BY THE HIGHER PLANTS. Bot. Zhur. SSSR (Jour. Bot. URSS) 18: 240–261. [In Russian. English Summary, p. 261.]
(11) NIGHTINGALE, G. T.
1937. THE NITROGEN NUTRITION OF GREEN PLANTS. Bot. Rev. 3: 85–174.
(12) SAKAMURA, T.
1930. DIE RESORPTION DES AMMONIUM- UND NITRATSTICKSTOFFS DURCH ASPERGILLUS ORYZAE. Planta 11: [765]–814.
(13) ———
1934. AMMONIO- UND NITRATOPHILIE BEI ASPERGILLUS ORYZAE IM BESONDEREN ZUSAMMENHANG MIT SCHWERMETALLEN. Jour. Faculty Sci. Hokkaido Imp. Univ. (5) 3: 121–138.
(14) STEINBERG, R. A.
1936. EFFECTS OF BARIUM SALTS UPON ASPERGILLUS NIGER AND THEIR BEARING UPON THE SULPHUR AND ZINC METABOLISM OF THE FUNGUS IN AN OPTIMUM SOLUTION. Bot. Gaz. 97: 666–671.
(15) ———
1936. SOME EFFECTS OF THE HEAVY METALS ESSENTIAL FOR THE NUTRITION OF ASPERGILLUS NIGER UPON ITS GROWTH. Amer. Jour. Bot. 23: 227–231.
(16) ———
1936 RELATION OF ACCESSORY GROWTH SUBSTANCES TO HEAVY METALS, INCLUDING MOLYBDENUM, IN THE NUTRITION OF ASPERGILLUS NIGER. Jour. Agr. Research 52: 439–448.
(17) TIEDJENS, V. A., and BLAKE, M. A.
1932. FACTORS AFFECTING THE USE OF NITRATE AND AMMONIUM NITROGEN BY APPLE TREES. N. J. Agr. Expt. Sta. Bul. 547, 32 pp., illus.

THE TRANSLOCATION OF DERRIS CONSTITUENTS IN BEAN PLANTS[1]

By ROBERT A. FULTON, *associate entomologist*, and HORATIO C. MASON, *assistant entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*[2]

INTRODUCTION

In certain field tests with insecticides for the control of the Mexican bean beetle (*Epilachna varivestis* Muls.) the growth of the bean plants that formed after the application of a suspension of derris or cube in water appeared to receive some protection from infestation. It was suspected that this protection was due to the adsorption-absorption and translocation of the derris or cube constituents. With this in mind tests were undertaken to determine whether this phenomenon could be explained.

GENERAL METHODS OF PLANT TREATMENT

All the bean plants were grown under greenhouse conditions. At the beginning of all tests the pots were washed with water to prevent contamination, and in the last series fresh soil and new pots and stakes were used.

As soon as the first true leaves had formed and the buds of the first trifoliate leaves were forming, the plants were treated with the derris suspension, some by spraying with a compressed-air hand sprayer, some by painting the first true leaves with a camel's-hair brush, and some by painting only the stems. After the treatment the plants were staked and intermingled among untreated plants, with sufficient space between pots to prevent contamination. All moisture for plant growth was supplied by adding water to the pot saucer or at the base of the plant.

Pinto beans were used in certain larval feeding tests (A and B, table 1), and extracts from the same variety were used in tests with goldfish (A and B, table 2). Burpee Stringless Green-Pod beans were used in all the remaining tests.

FEEDING TESTS WITH MEXICAN BEAN BEETLE LARVAE

In all larval feeding tests but one the leaves were removed from the bean plants, and the feeding was conducted in a constant-temperature cabinet operated at 26.7° C. and 60-percent relative humidity, by a technique developed for toxicological studies of the Mexican bean beetle larvae.[3] The leaves were laid flat on a glass plate approximately 10 by 10 inches, with the lower surfaces upward and with the stems inserted in individual water reservoirs. An open glass feeding cell,

[1] Received for publication Aug. 14, 1937; issued February 1938.
[2] The authors wish to acknowledge many helpful suggestions from N. F. Howard and W. H. White during the course of this investigation.
[3] FULTON, R. A. A TECHNIQUE FOR THE TOXICOLOGICAL STUDIES OF MEXICAN BEAN BEETLE LARVAE. (In preparation.)

approximately 1¼ inches in diameter by 1¼ to 1½ inches high, was placed on each leaflet. One second-instar Mexican bean beetle larva, reared under laboratory conditions, was placed in each cell, except in the second test (B, table 1) when two larvae were used. They were confined by means of a screen-wire top. The plate was then placed in the constant-temperature cabinet.

In one test (A, table 1) the feeding was done on the plant under greenhouse conditions. The cells were placed on the leaves with the under surfaces upward, each leaflet resting on a small wooden block supported by a wire stirrup. One larva was placed in each cell and confined as described above. Third- and fourth-instar larvae collected from the field were used, but the larvae were in only fair condition, as it was late in the fall when the test was conducted.

In all tests the larvae were allowed to feed for a definite time, at the end of which examinations were made and mortality and area of leaf surface consumed were recorded. The leaf surface removed by each larva was determined by placing a "flexible glass" guide marked in 2-mm squares directly over the leaf. The larvae were transferred to new leaves when necessary, and to prevent contamination during the transfer, hands were washed with soap and water and clean camel's-hair brushes were used for each type of treatment.

The results of these tests are summarized in table 1.

TABLE 1.—*Feeding of Mexican bean beetle larvae on new growth of bean plants previously treated with a suspension of derris in water* [1]

Test	Type of treatment	Trifoliate leaves used	Time after treatment when feeding tests were begun	Larvae used	Duration of feeding tests	Average total leaf area consumed per larva	Reduction of feeding area compared with that of untreated plants	Mortality
			Days	*Number*	*Hours*	*Sq. millimeters*	*Percent*	*Percent*
A	Plants sprayed	First	12	16	74	421	51	0
	Untreated plants	do		[2] 4	74	866		0
B	Plants sprayed	do	12	30	72	143	24	6.6
	Leaves painted	do	12	24	72	115	39	8.3
	Stems painted	do	12	24	72	125	34	4.2
	Untreated plants	do		24	72	188		4.2
C	Leaves painted	do	11	15	144	687	37	0
	Stems painted	do	11	15	144	761	30	6.6
	Untreated plants	do		15	144	1,092		0
D	Leaves painted	Second	18	15	96	194	42	0
	Stems painted	do	18	15	96	220	34	0
	Untreated plants	do		15	96	335		0
E	Leaves painted	Third	27	15	96	267	32	6.6
	Stems painted	do	27	15	96	278	29	0
	Untreated plants	do		15	96	393		0

[1] In test A the rotenone content of the derris suspension was 0.025 percent; in all other tests the rotenone content of the suspension was 0.05 percent.
[2] 1 larva escaped during the first 24 hours.

The data show that the average leaf area removed per larva on new growth of treated plants is definitely reduced, as compared with the feeding area removed from untreated plants. Reductions in feeding area, 51 and 24 percent, respectively, occurred when first trifoliate leaves from sprayed plants were used (tests A and B). There were reductions of 39 and 37 percent, respectively, in the feeding on first trifoliate leaves from leaf-painted plants (tests B and C). When new

growth from stem-painted plants was used, the average reductions in leaf area consumed were 34 and 30 percent, respectively (tests B and C). When second trifoliate leaves from the same plants used in the preceding tests were fed (test D), reductions of 42 percent on leaf-painted and 34 percent on stem-painted plants occurred. Third trifoliate leaves from the same group of plants used in the two preceding tests were fed (test E), and even though the original treated area was much less in proportion to the total area of new growth after treatment, reductions of 32 and 29 percent occurred on leaf-painted and stem-painted plants, respectively.

When the daily totals were compared, it was noted that the area consumed per larva from leaf-painted plants was consistently higher during the first 24 hours. In the case of the stem-painted plants the area consumed on the first day was smaller than on the days of subsequent observations (after 2 to 4 days). At the close of the tests the total area consumed was greater on stem-painted plants than on leaf-painted plants.

TOXICITY TESTS WITH GOLDFISH

For the biological tests with goldfish (*Carassius auratus*) extracts were prepared from leaves of the same plants that were used in the feeding tests. This was done by extracting the leaf material with chloroform to eliminate excessive chlorophyll in the final preparation. The chloroform extract was evaporated to dryness on a steam bath and the residue steeped with an excess of acetone. The acetone solution was then evaporated and a known quantity of acetone added to this residue.

The tests with goldfish were made in a manner similar to that described by Gersdorff.[4] Aliquots of the acetone solution were added to known quantities of water at the rate of 1 cc per liter. In the first test recorded (A, table 2) 1,300 cc of water was used, and the solution was divided between two jars, 650 cc in each. Three fishes were placed in each jar. In the remaining tests 1,200 cc of water was used, divided between two jars, 600 cc in each, and two fishes were placed in each jar. The jars were then placed in a constant-temperature cabinet operating at 26.7° C. Observations were made at 30-minute intervals and the time of death was recorded. The results are shown in table 2.

In the first test (A) leaves were removed from the new growth of plants previously painted with a suspension of derris in water. The leaves were dried and an extract was prepared from 350 g of the material. This extract killed all the fishes in an average time of 220 minutes. The fishes in the solution prepared from dried leaves of the new growth of untreated plants were all alive and appeared normal at the end of 1,320 minutes.

Extracts prepared from first trifoliate leaves of leaf-painted and stem-painted plants (test B) killed all the fishes used in average times of 135 and 165 minutes, respectively. All the fishes were alive after 1,620 minutes in the solution prepared from untreated plants.

A third test (C) was performed to determine whether toxic material could be detected in the first trifoliate leaves by crushing the leaves and macerating them with water. The water extract was drained,

[4] GERSDORFF, W. A. A METHOD FOR THE STUDY OF TOXICITY USING GOLDFISH. Jour. Amer. Chem. Soc. 52: 3440-3445, illus. 1930.

filtered through cotton, and then made up to the same volume as used in other tests. All fishes were dead in the water extract of leaves from leaf-painted plants in an average time of 445 minutes and in the water extract of stem-painted plants in 482 minutes. All fishes in the water extract from leaves of untreated plants appeared normal at the end of 1,340 minutes.

TABLE 2.—*Toxicity to goldfish of extracts of new growth of bean plants previously treated with a suspension of derris in water* [1]

Test	Type of treatment	Trifoliate leaves used for extracts	Weight of leaves	Time after treatment when toxicity tests were begun	Goldfish used	Average weight of goldfish	Average time before death	Mortality
			Grams	*Days*	*Number*	*Grams*	*Minutes*	*Percent*
A [2]	Leaves painted	First	350	26	6	3.7	220	100
	Untreated plants	do	257		6	2.3		0
B	Leaves painted	do	87.5	11	4	1.91	135	100
	Stems painted	do	97.6	11	4	2.77	165	100
	Untreated plants	do	93.5		4	2.12		0
C [3]	Leaves painted	do	94	11	4	2.46	445	100
	Stems painted	do	89	11	4	2.18	482	100
	Untreated plants	do	65		4	2.56		0
D	Leaves painted	Second	114	18	4	1.78	105	100
	Stems painted	do	116	18	4	1.64	202	100
	Untreated plants	do	123		4	1.18		0
E	Leaves painted	Third	87	27	4	1.41	112	100
	Stems painted	do	92	27	4	1.86	462	100
	Untreated plants	do	85		4	1.45		0

[1] Derris suspension of 0.05 percent rotenone content was used in all tests except A, where a suspension of 0.25 percent rotenone was used.

[2] Leaves were weighed dry; in all other tests they were weighed green. In this test all the treated leaves had dropped from the plants when untreated leaves were picked.

[3] Solutions prepared by crushing the leaves in distilled water.

Extracts prepared from second trifoliate leaves (test D) of the same plants used in the two preceding tests killed all the fishes in the leaf-painted and stem-painted solutions in 105 and 202 minutes, respectively. At the end of 420 minutes all fishes in the untreated plant extract appeared normal.

In the final test with goldfish (E) third trifoliate leaves were removed from the same plants that were used in the three preceding tests. All the fishes were dead in an average time of 112 minutes in the extract from leaf-painted plants, whereas in the extract from stem-painted plants all were not dead until after an average of 462 minutes. All fishes in the untreated plant extract appeared normal at the end of 1,130 minutes.

The results recorded above show that toxic ingredients are present in the new growth of plants previously treated with suspensions of derris in water.

ISOLATION AND IDENTIFICATION OF ROTENONE

In one case rotenone was actually isolated from the plant material. Eight hundred grams of dry leaves from the new growth that formed after the application of a suspension of derris in water (0.025 percent of rotenone) was extracted with chloroform. The dried leaf material was covered with chloroform and allowed to stand at room temperature in subdued light for 3 days. At the end of that time the chloroform

solution was removed and the leaf material was again covered with chloroform. By using a known weight of leaf material, it had previously been determined that the cold method of extraction reduced the chlorophyll content of the extract. The chloroform extracts were evaporated to dryness and the residue was removed with carbon tetrachloride. The carbon tetrachloride solution was allowed to stand for several days in an ice box maintained at approximately 4° C. At the end of that time small needlelike crystals appeared in the solution. These crystals were washed with a small quantity of cold carbon tetrachloride, recrystallized from acetone to convert any solvate present to rotenone, and the melting point was then determined. Only 8 mg of this material was recovered from 800 g of dried new-growth leaves. The crystals gave the characteristic test with the Gross-Smith [5] colorimetric method, and had a melting point of 163.5° C., or approximately the melting point of rotenone (163° C.). The crystals were also tested by the use of the blue color test of Durham and they gave a color similar to that produced by bromothymol blue indicator at pH 7.2.

One milligram of this material was added to 650 cc of water containing 0.6 cc of acetone as a dispersing agent and tested with three goldfish at 26.7° C. All the fishes were killed in an average time of 77 minutes.

SUMMARY AND CONCLUSION

A definite retarding in the feeding of Mexican bean beetle larvae has been observed on the new growth of bean plants that have been treated with derris powder. First, second, and third trifoliate leaves, formed after the first pair of true leaves had been treated, were found to be less palatable to bean beetle larvae than similar leaves from untreated plants.

Extracts prepared from first, second, and third trifoliate leaves formed after the application of derris powder to the first pair of true leaves caused 100-percent mortality to goldfish. Extracts prepared from first, second, and third trifoliate leaves from untreated plants were not toxic.

A water extract prepared by macerating new growth of bean plants that had been treated with derris was fatal to goldfish.

In one case, where a large quantity of new-growth material was available, a crystalline substance resembling rotenone was isolated. The material when purified had a melting point of 163.5° C., gave the characteristic color with the Gross-Smith method, and the blue color test of Durham. One milligram of this substance in 650 cc of water at 26.7° C. killed three goldfish in an average of 77 minutes.

These experiments demonstrate that derris constituents are translocated from the outer surfaces of leaves to first, second, and third trifoliate leaves formed after the application of derris powder in water suspension to the first true leaves and stems of bean plants.

[5] GROSS, C. R., and SMITH, C. M. COLORIMETRIC METHOD FOR THE DETERMINATION OF ROTENONE. Jour. Assoc. Off. Agr. Chem. 17: 336–339. 1934.

QUANTITATIVE INJECTION AND EFFECTS OF NICOTINE IN INSECTS[1]

By N. E. McIndoo

Senior entomologist, Division of Control Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

Nicotine is used as an insecticide chiefly for the control of small, soft-bodied, sucking insects. As ordinarily applied in sprays or dusts, it is ineffective against many species of caterpillars, beetles, flies, etc., either because it does not reach the vital parts of these insects or because it is not sufficiently toxic to them. It is therefore of interest to know whether certain species that are considered resistant to nicotine are actually susceptible when this material is introduced by injection into the body cavity. This study dealt with the improvement of apparatus for the quantitative injection of solutions into insects, the comparative susceptibility of various species to nicotine, the effect of age of insects on susceptibility, the difference between the toxicity of nicotine and that of nicotine sulphate, and other toxicological problems.

INJECTION APPARATUS, MATERIAL, AND METHODS

An attempt was made to use apparatus more accurate and more easily handled than any yet designed to feed or to inject quantitative doses into insects. A micrometer syringe (fig. 1, *A*), described by Trevan (*7*)[2], was used apparently for the first time to inject measured volumes of an insecticide into insects, although Storey (*6*) had previously used it to inject plant viruses into leafhoppers. This apparatus consists simply of an ordinary hypodermic syringe (*s*), the plunger of which is actuated by the head (*h*) of the micrometer (*m*), which is fastened to the syringe by a clamp (*c*). It was calibrated with distilled water in the Volumetric Section of the Bureau of Standards. A movement of the micrometer head through five divisions delivered 0.00124 cc (1.24 mm^3) with an error of less than 4 percent. A 27-gage steel hypodermic needle (*n*) 30 mm long was used on the syringe.

Various means of mounting and manipulating the syringe were tried. The arrangement illustrated in figure 2, *A*, was adopted, the syringe (*s*) being rigidly clamped in a horizontal position on the pillar (*mp*) of a dissecting microscope. The syringe was moved up or down by rack and pinion and in a horizontal plane by pushing the microscope base (*mb*) with both hands. Under the micrometer head (*h*) a wooden support (*ws*) was mounted to steady the hand while turning the micrometer screw.

[1] Received for publication August 4, 1937; issued February 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 921.

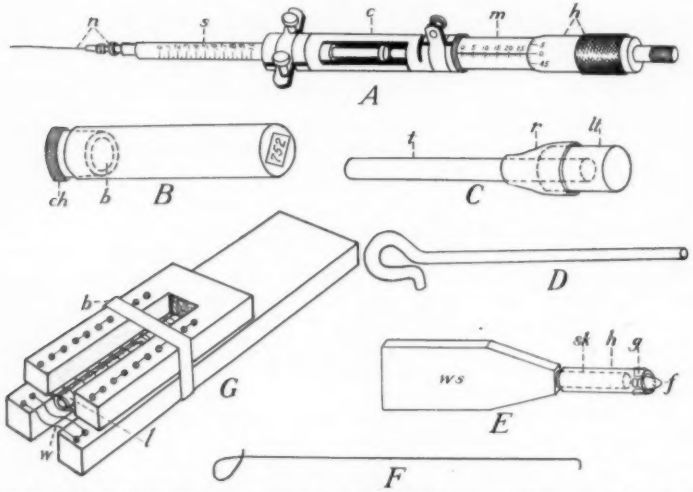

FIGURE 1.—Micrometer syringe and other devices used to inject insects: *A*, Micrometer syringe showing hypodermic needle (*n*), syringe (*s*), clamp (*c*), micrometer (*m*), and micrometer head (*h*); *B*, shell vial closed with a hollow cork stopper (*b*) whose outer end is covered with cheesecloth (*ch*); *C*, device to remove flies from vials, consisting of small glass tube (*t*), larger glass tube (*lt*), and rubber tube (*r*); *D*, steel rod to transfer flies; *E*, device to hold fly (*f*) during injection, consisting of wooden support (*ws*), its shank (*sk*), glass holder (*h*), and gummed cloth (*q*) to constrict opening in holder; *F*, wire to remove fly from glass holder; *G*, device to hold larva (*l*) during injection, showing fine wire grids (*w*) and rubber band (*b*) to hold the frames together.

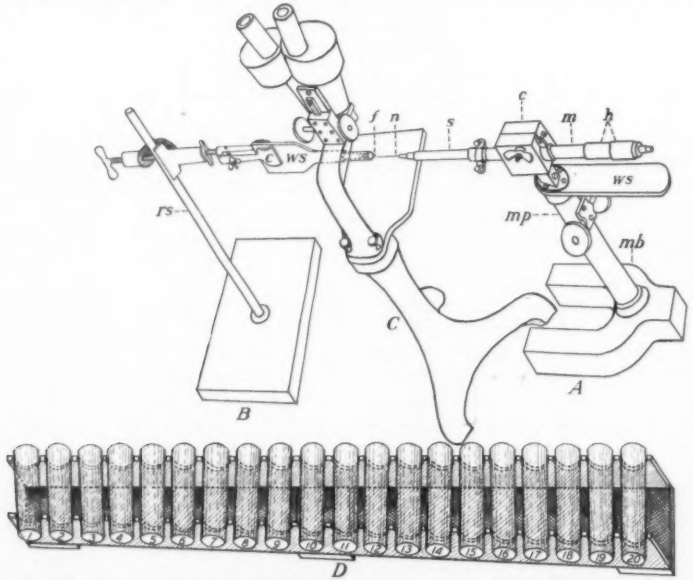

FIGURE 2.—Apparatus used to inject flies and containers for them: *A*, Micrometer syringe mounted on pillar (*mp*) of dissecting microscope, showing base (*mb*) of microscope, wooden support (*ws*), needle (*n*), syringe (*s*), wooden clamp (*c*) to hold syringe, micrometer (*m*), and micrometer head (*h*); *B*, ring stand (*rs*) and clamp (*c*) to hold wooden support (*ws*) and fly (*f*); *C*, binocular microscope; *D*, rack and shell vials in which the treated flies were kept.

Adults and larvae of the green-bottle blowfly (*Lucilia sericata* Meig.), the black blowfly (*Phormia regina* Meig.), and the blue-bottle blowfly (*Calliphora erythrocephala* Meig.) were used in most of the experiments. They were reared in the manner already described by the writer (*4*). Mature or nearly mature larvae of the following lepidopterous species were used in other tests: Eastern tent caterpillar (*Malacosoma americana* (F.)), codling moth (*Carpocapsa pomonella* L.), silkworm (*Bombyx mori* L.), and southern armyworm (*Prodenia eridania* (Cram.)). Nearly 4,000 insects were injected, and when large numbers of a species were used for a single solution the figures were treated statistically.

Solutions of pure nicotine and of nicotine sulphate in distilled water were prepared for the writer in the Division of Insecticide Investigations. These solutions were diluted with distilled water by the writer to make the following percentage concentrations of nicotine: 4.0, 3.0, 2.0, 1.5, 1.0, 0.8, 0.5, 0.25, 0.125, 0.062, and 0.031. A solution of each concentration was stored in a small bottle wrapped with black paper. Distilled water was used for check injections.

Shortly before a series of injections a sufficient number of ventilated, stoppered vials (fig. 1, *B*), 18 by 75 mm, were weighed collectively. The flies were then caught, one fly of known sex to a vial, by placing an unstoppered vial over the desired fly in the rearing cage. The stoppered vials were weighed again, and the average weight of a fly was calculated. A set of flies consisted of 20 individuals, usually 10 males and 10 females. The vials holding them were placed on a rack, shown in figure 2, *D*.

To inject a fly it was necessary to hold it mechanically under a binocular microscope (fig. 2, *C*). This was done as follows: The fly was removed from its vial by means of a glass tube (fig. 1, *C*). The larger tube (*lt*) was inserted into the vial, and the fly was made to pass through it into a smaller tube (*t*), from which it was forced by a steel rod (*D*) into a glass holder (*E*, *h*). The shank (*E*, *sk*) of a wooden holder (*ws*) was finally thrust full length into the holder, thus forcing the abdomen of the fly (*f*) through the constricted end to the exterior. Two sizes of holders were used, according to the size of the fly injected. Similar holders for queen bees have been described by Nolan (*5*, *p. 21*).

The flies were usually injected on the left side between the second and third abdominal segments halfway between the ventral and dorsal midlines. The needle was inserted the width of the second segment, allowed to remain for 15 seconds, and then pulled out slowly. The fly was immediately removed from the holder by a wire with a hooked end (fig. 1, *F*) and placed in its vial. About an hour was required to inject 20 flies. Each vial, containing an injected fly and sugar solution on cotton, was replaced on the rack for observation. During the period of injection the temperature of the vials was held at about 29° C., but thereafter it was uncontrolled and fluctuated with room temperature. Hockenyos and Lilly (*1*) determined that temperature was not an important factor when they injected nicotine into caterpillars.

During injection the larger larvae were held between two wooden frames with wire grids (fig. 1, *G*, *w*). The lower frame was extended beyond the grid to provide a means for clamping it to the ring stand. A larva was placed on the grid of the lower frame, and the grid of the

upper frame was placed over it. The two frames were held together with a rubber band (*G, b*) so placed that the larva was not injured by compression but was prevented from squirming loose. Smaller larvae were held between the grid of the upper frame and the extension of the lower frame. The needle of the syringe was inserted in the side of the larva between the fourth and fifth abdominal segments, counting from the posterior end. To prevent bleeding, the needle was left in the insect for 1 minute and then gently removed. The injected larvae were given food and confined for observation in small wire-screen cages.

Several kinds of records were taken. Longevity records, or counts of the number of dead insects, were made at 4 p. m., and three times daily thereafter, at 9 a. m., 12 m., and 4 p. m., until all the insects were dead. In some cases, for the sake of greater accuracy, counts were made hourly. The average length of life of the insects in the set was then calculated. At the same time activity records were taken on the following basis: No movement was represented by 0, very slight movement by 1, slight movement 2, considerable movement (able to stand on feet) 3, fair activity 5, nearly normal activity 7, and normal activity 10. These figures were then averaged on a percentage basis. Insects that were apparently lifeless were warmed under an electric lamp to bring out any latent activity. Finally, a paralysis record was kept to measure the duration of paralyzation; it included the first-reaction time, or the period after injection before the insect showed the first signs of life, and the revival time, or the period after injection before the fly was able to get upon its feet.

COMPARATIVE SUSCEPTIBILITY OF VARIOUS SPECIES OF INSECTS

A long series of tests was conducted to determine the comparative susceptibility, on the basis of body weight, of various species and the sexes to nicotine. Both nicotine and nicotine sulphate were injected, but the quantities are expressed as pure nicotine. An effort was made to keep the activity between 5 and 6 percent and the longevity, based on 100-percent mortality, at about 24 hours. The susceptibility was expressed as the body weight of the insect that was killed by 1 mg of pure nicotine.

The data for these tests are given in table 1. Since 2,727 mg of *Lucilia* males and 302 mg of *Phormia* larvae were killed by 1 mg of nicotine, the flies were about nine times as susceptible as the larvae. *Lucilia* males were considerably more susceptible than *Lucilia* females; *Calliphora* males and females were practically the same, but much less susceptible than *Lucilia*; codling moth larvae were about equal in susceptibility to *Lucilia* females; and the other larvae were much less susceptible than any of these insects. It is difficult, and sometimes impossible, to kill the southern armyworm by applying nicotine compounds to foliage, and the figure given in the table bears this out. The susceptiblity of the larval forms, except that of the codling moth, was about half that of the imaginal forms.

Although 120 tent caterpillars were also used in these tests, the figures are not included in table 1. If the dosage given to them had been doubled, their susceptibility would probably have been similar to that of the silkworm.

TABLE 1.—*Comparative susceptibility of various species of insects and their sexes to nicotine, based on 100-percent mortality*

Species and sex	Insects	Average weight of insects	Nicotine injected per insect	Average longevity	Average activity	Body weight of insects killed by 1 mg of nicotine
	Number	*Mg*	*Mg*	*Hours*	*Percent*	*Mg*
Lucilia, males	100	27	0.0099	23.7	5.4	2,727
Lucilia, females	90	38	.0248	26.4	6.1	1,532
	90	42	.0372	24.5	5.2	1,129
Calliphora, males	100	69	.0744	21.6	5.0	927
Calliphora, females	100	77	.0843	26.1	5.3	913
Codling moth larvae	60	44	.0372	20.3	6.1	1,182
Silkworms (fourth-instar)	60	517	.7440	27.6	6.1	695
Southern armyworms	20	625	1.2400	40.3	6.7	504
Phormia larvae (full-grown)	40	75	.2480	21.3	5.7	302

Numerous control tests, in which distilled water was used, were likewise conducted. Doses of it equal to those of the insecticides had little or no effect on the insects. The longevity was practically the same as that of untreated insects and the activity was 100 percent or nearly so.

EFFECT OF AGE ON SUSCEPTIBILITY OF FLIES

Earlier tests indicated that the age of the injected flies affected their susceptibility to nicotine, and that if more consistent results were to be obtained greater attention would have to be given to the age of the insects treated. A series of tests was therefore conducted with 20 *Phormia* flies, 10 males and 10 females, of various ages. A dose of 1.24 mm^3 of 3-percent nicotine solution was first tried to see if curves could be drawn representing the daily toxicity, based on longevity and activity records, during the lifetime (24 days or more) of the flies. This concentration was too high, however, for the curves (fig. 3, *a* and *b*) quickly ascended from the first to the fourth day and then quickly descended to the sixth day. A dose of 1.24 mm^3 of a 2-percent solution was next tried. This concentration produced curves (*c* and *d*) which rose quickly to the fifth day, but descended less rapidly to the twelfth day, and thereafter it was too high, for the activity had reached 3.3 percent and the longevity had reached 17 hours, both of which figures were the lowest that could be recorded on the scale used. After the twelfth day 0.5- and 0.25-percent solutions were used, and the average longevity was 17 hours on the fourteenth and eighteenth days, respectively. Flies 14 days old were therefore killed with 0.0031 mg of nicotine (0.25-percent solution) in 17 hours, while flies 5 days old were killed with 0.0248 mg (2-percent solution) in 22.2 hours. The old flies were more than eight times as susceptible as the young ones. The control insects, injected with distilled water, were affected little or not at all, even after the twelfth day, while those injected with nicotine were quickly paralyzed and soon died.

The flies were most resistant on the fourth and fifth days, but thereafter their susceptibility increased with age. Upon emerging as adults the flies were weak, but they gradually gained strength and

on the fourth day began to lay eggs. This information may explain why the curves ascend from the first to the fifth day, but the increasing age of the flies certainly explains why the curves descend thereafter.

The comparative susceptibility of *Phormia* flies of different ages to various concentrations of nicotine was also studied from hourly longevity records. In this case the susceptibility ratio was obtained by dividing the body weight of the insects by the weight of nicotine

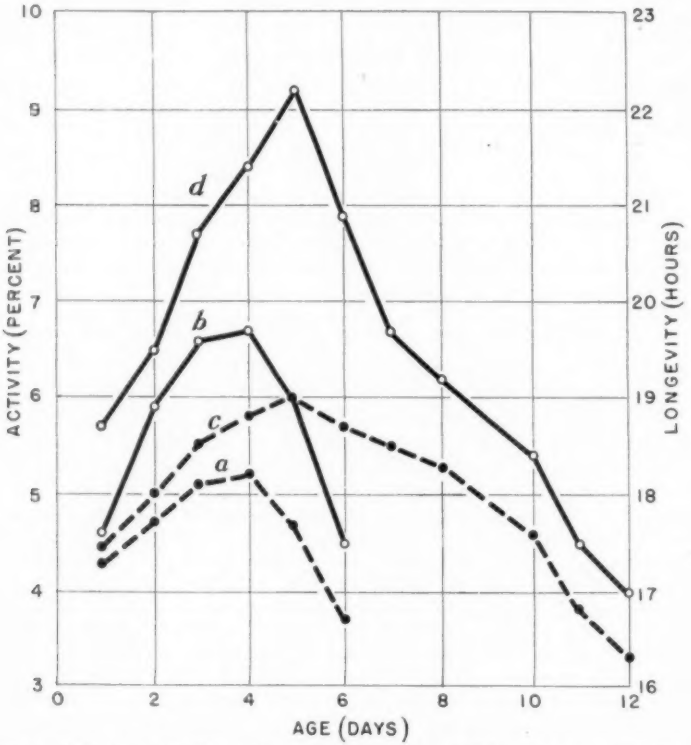

FIGURE 3.—Effect of age of flies (*Phormia*) on toxicity of nicotine: *a*, Activity, and *b*, longevity, with 3-percent solution; *c*, activity, and *d*, longevity, with 2-percent solution.

injected and then dividing this quotient by the longevity. The results are shown in table 2. Flies 5 days old are shown to be more resistant than flies 3 days old. Flies 5 days old showed no difference in toxic effects when injected with the same quantity of nicotine in 1- and 3-percent solutions. Both the longevity figures and the susceptibility ratios show that the males were more susceptible than the females. On the basis of milligrams of nicotine injected, the 16-day-old flies were six times as susceptible as the 3- and 5-day-old flies, whereas on the basis of the susceptibility ratios they were nearly seven times as susceptible.

TABLE 2.—*Comparative susceptibility of Phormia flies of different ages to various concentrations of nicotine, based on hourly longevity records*

Age of flies (days)	Weight of flies		Nicotine solution injected			Longevity of flies			Ratio of susceptibility	
	Females	Males	Concentration	Dose	Content of pure nicotine	Females	Males	Average	Females	Males
	Mg	*Mg*	*Percent*	*Mm³*	*Mg*	*Hours*	*Hours*	*Hours*		
3	42	38	3	1.24	0.0372	9.3	6.5	7.9	121	157
5	45	40	3	1.24	.0372	9.6	8.3	8.9	126	130
5	45	40	1	3.72	.0372	9.5	8.0	8.8	127	134
16	50	------	.5	1.24	.0062	9.5	------	------	849	------

COMPARISON OF TOXICITIES OF NICOTINE AND OF NICOTINE SULPHATE

Early tests with 2- and 3-percent solutions indicated that nicotine was more toxic to *Lucilia* than nicotine sulphate. On the basis of 100-percent mortality the activity records showed the free nicotine to be 14 percent more toxic than the nicotine salt, whereas the longevity records showed it to be only 8 percent more toxic. On the basis of 50-percent mortality the nicotine solution was 8.4 percent more toxic, according to the longevity records. From later activity records based on 100-percent mortality it was determined statistically that the nicotine solutions were not more toxic to female *Lucilia* than the nicotine sulphate solutions. The methods used allowed too many large deviations, and later it was ascertained that the nicotine solutions were the more toxic. In all instances the solutions of nicotine and nicotine sulphate were prepared on the basis of equal nicotine content.

In the hope of getting more accurate results, the toxicities of nicotine and nicotine sulphate were compared by the paralysis method. A dose of 1.24 mm^3 of 0.25-percent solution was injected into each fly of five sets of *Phormia* females and two sets of *Phormia* males. For the males the average first-reaction time for nicotine was 17.9 minutes and for nicotine sulphate 15.8 minutes; the average revival time for nicotine was 44.9 minutes and for nicotine sulphate 42.6 minutes. These figures show nicotine to be the more toxic.

The data for the females are given in table 3. According to the first-reaction time nicotine is 22.2 percent more toxic than nicotine sulphate, but according to the revival time it is 26.5 percent more toxic. These figures may be considered reliable, because, according to the statistical test, the probability of such differences occurring by chance is only 1 in 100.

The pH values of the solutions of nicotine and nicotine sulphate differed. Hockenyos and Lilly (*1*) determined that the effect of varying the acidity was very slight. In control tests the writer used potassium hydroxide and hydrochloric acid in distilled water and concluded that hydrogen-ion concentration alone had little effect. Levy's saline solution was slightly better than distilled water for control tests, but insecticides are never dissolved in it. Other tests in which large volumes of distilled water were injected into flies showed that the insects occasionally tolerated a dose equal to one-fourth the body weight.

TABLE 3.—*Difference between toxicities of nicotine and of nicotine sulphate to Phormia females as determined from paralysis records*

Group no.	First-reaction time		Revival time	
	Nicotine	Nicotine sulphate	Nicotine	Nicotine sulphate
	Minutes	*Minutes*	*Minutes*	*Minutes*
1	13.4	11.6	47.3	33.2
2	14.6	13.1	44.9	32.7
3	16.1	13.2	35.9	25.0
4	18.4	11.3	33.4	30.4
5	14.4	13.7	32.1	31.9
Mean [1]	15.5	12.6	38.7	30.7
Difference		2.8 ± 1.24		8.1 ± 2.59
Probability		.01		>.01
Total samples—number	46	50	43	46

[1] Mean of total number of samples in the five groups.

COMPARISON OF TOXICITIES OF NICOTINE IN DISTILLED WATER AND OF NICOTINE SULPHATE IN POTASSIUM HYDROXIDE SOLUTION

To free the nicotine in the 0.25-percent nicotine sulphate solution, a 0.1-percent solution of potassium hydroxide was used instead of distilled water to reduce the 20-percent solution of nicotine sulphate to this dilution. In controls a 0.1-percent solution of potassium hydroxide produced no signs of paralysis. In comparative tests five sets of *Phormia* males and females were injected with this solution and with a 0.25-percent solution of nicotine in distilled water. The results are given in table 4. The differences between the average results obtained were slight and not significant. The freed nicotine in the nicotine sulphate solution was, therefore, equally as toxic as the other free-nicotine solution.

TABLE 4.—*Difference between toxicities to Phormia males and females of nicotine in distilled water and of nicotine sulphate in potassium hydroxide solution as determined from paralysis records*

Group no.	First-reaction time		Revival time	
	Nicotine	Nicotine sulphate	Nicotine	Nicotine sulphate
	Minutes	*Minutes*	*Minutes*	*Minutes*
1	13.2	14.6	40.4	38.4
2	9.4	11.8	39.0	39.7
3	15.3	13.4	43.4	41.7
4	13.3	19.8	43.2	37.6
5	14.2	14.9	37.8	40.1
Mean [1]	13.1	14.9	40.8	39.5
Difference		1.8±1.06		1.3±2.96
Probability		>.20		>.60
Total samples—number	50	50	42	42

[1] Mean of total number of samples in the five groups.

A STUDY OF SYNERGISM

Levine and Richardson (*2*) made a study of synergism by injecting into cockroaches nicotine solutions made up in 0.1-molar solutions of

chlorides and bicarbonates of potassium and sodium and in distilled water (the control). Three of these salts increased the toxicity of nicotine, but potassium chloride was the best for this purpose. The writer compared the toxicity of nicotine solutions in distilled water and in 0.1-molar potassium chloride.

Three sets of *Calliphora* females, 13, 14, and 15 days old, were injected with 0.25-percent solutions, the dose being 2.48 mm^3, or 0.0062 mg of pure nicotine. The solutions were prepared by diluting one portion of the 20-percent nicotine solution with distilled water and another portion with 0.1-molar potassium chloride. The potassium chloride solution, used alone as a control, caused no sign of paralysis. As judged by the revival time, which is more reliable than the first-reaction time, the nicotine diluted with the potassium chloride solution was 10.4 percent more toxic than that diluted with distilled water, but when the figures, including many large deviations, were treated statistically there was no significant difference (table 5). If 100 flies, instead of 30, had been injected with each solution, the difference between the means might have been significant.

TABLE 5.—*Difference between toxicities to Calliphora flies of nicotine dissolved in distilled water and of nicotine dissolved in 0.1-molar potassium chloride solution*

Group no.	First-reaction time		Revival time	
	Nicotine in distilled water	Nicotine in potassium chloride solution	Nicotine in distilled water	Nicotine in potassium chloride solution
	Minutes	*Minutes*	*Minutes*	*Minutes*
1	11.3	10.3	37.1	43.3
2	10.6	12.0	39.3	40.8
3	11.2	9.5	39.1	43.4
Mean [1]	11.0	10.6	39.5	42.5
Difference		0.4±1.12		4.0±5.74
Probability		.60		.40
Total samples—number	30	30	28	28

[1] Mean of total number of samples in the 3 groups.

A STUDY OF MOTOR PARALYSIS

To ascertain more accurately the effects of nicotine on flies, the paralysis method was further used in a study of motor paralysis. In the first series of tests the reactions of *Phormia* flies 2 to 6 days old were compared with those of *Phormia* flies 23 to 30 days old. The first-reaction time and the revival time of the young flies did not differ greatly among themselves, but they did differ widely from those of the old flies. All the injected flies were totally paralyzed, but the period of paralyzation depended on the percentage of nicotine used and the age of the flies. The old flies were the more easily paralyzed and the paralysis lasted longer.

Tests were also made with six sets of *Phormia* flies ranging in age from 9 to 16 days. Flies of the age indicated were injected with doses of 1.24 mm^3 of nicotine solution of the following concentrations: 0.031 (9 days), 0.062 (10 days), 0.125 (11 days), 0.25 (12 days), 0.5 (15 days), and 1 percent (16 days). The weights of pure nicotine in these doses were 0.0004, 0.0008, 0.0016, 0.0031, 0.0062, and 0.0124

mg. The curves for first-reaction time (*a*) and revival time (*b*) in figure 4 start near 0 minutes when the smallest dose was used, and gradually ascend as practically straight lines. The curve for the first-reaction time is continued the full distance, but that for the revival time runs only one-fourth of the distance, because most of the flies failed to revive when they were given doses of 0.0062 and 0.0124 mg. Increased age caused these lines to ascend slightly, but increased dose caused most of the rise, as shown in previous tests.

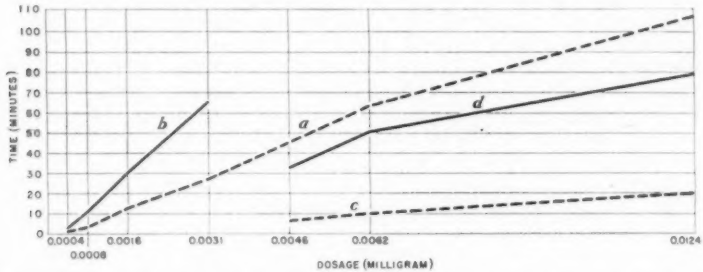

FIGURE 4.—Effect of increasing doses of nicotine on first-reaction time and revival time: *a*, First-reaction time, and *b*, revival time, of *Phormia*; *c*, first-reaction time, and *d*, revival time, of *Calliphora*

Three sets of *Calliphora*, 7, 8, and 10 days old, were injected, respectively, with 2.48 mm³ of 0.125-, 0.25-, and 0.5-percent nicotine solutions. Since this dose of the 0.125-percent solution paralyzed only half the females, a 3.72-mm³ dose was then used. The weights of pure nicotine in this dose were 0.0046, 0.0062, and 0.0124 mg. Again the curves (fig. 4, *c* and *d*) gradually ascend as practically straight lines; and since the three sets of flies were of nearly the same age, increasing age had practically no influence on these curves. Table 6 shows that the males were always more susceptible to nicotine than the females. The susceptibility ratio in this case was obtained by dividing the weight of the flies by the weight of nicotine injected, multiplying this quotient by revival time, and dividing this product by 1,000.

TABLE 6.—*Susceptibility of Calliphora flies to nicotine as shown by a study of motor paralysis*

Nicotine injected (mg)	Sex of flies	Weight of flies	First-reaction time	Revival time	Ratio of susceptibility
		Mg	*Minutes*	*Minutes*	
0.0046	Females	60	1.4	10.4	136
	Males	50	11.2	55.7	605
	Average		6.3	33.0	
.0062	Females	60	9.6	47.0	455
	Males	50	10 9	64.8	522
	Average		10.2	55.9	
.0124	Females	67	13.6	57.7	312
	Males	60	24.1	100.0	484
	Average		18.9	78.8	

SYMPTOMS OF NICOTINE POISONING

In 1916 the writer (*3*) gave the first detailed description of nicotine poisoning in insects (bees). The information given here supplements that description.

When flies (*Phormia*) were totally paralyzed, the following symptoms were observed: About a second after the injection, while a fly was still in the glass holder, the abdomen, legs, and wings ceased moving vigorously, but quivered. The legs were usually folded together and the wings were bent toward the body. When a fly was quickly transferred to its vial, it often buzzed around on its back and kicked its legs, but the vigorous movements soon ceased, and then the wings were usually drawn to the body and the legs were folded together. The proboscis was always extended for a short time, then usually retracted, but it was common for a fly to die with its proboscis extended. A droplet of liquid often appeared on the proboscis and at the anus.

The following records pertain to injected larvae of various species: About 5 seconds after the tent caterpillars had been injected, convulsions occurred near the wounds and lasted half a minute. Later the larvae were completely paralyzed. When distilled water was used as a control, there were no convulsions and no signs of paralysis. Many of the injected silkworms spewed at the mouth and voided feces and liquid at the anus. Only slight movements were seen in the injected segments, but the integument often turned brownish or even darker. Movements of the same kind were also seen in the injected segments of southern armyworms. Codling moth larvae did not exhibit convulsions but only wriggled feebly. *Phormia* larvae showed only weak muscular movements near the needle, which was inserted at some distance from the visible heart. The nicotine did not seem to affect the heartbeat.

EFFECT OF POINT OF INJECTION ON TOXICITY

Hockenyos and Lilly (*1*) found that the toxicity of nicotine increased as the point of injection approached the head, where the largest ganglia occur. To ascertain whether the same is true in regard to the abdominal ganglia, three groups of 10 *Phormia* females, 13 days old, were injected between the second and third abdominal segments with 0.25-percent nicotine solution, the dose being 1.24 mm^3. Flies of the first group were injected on the ventral midline, those of the second on the dorsal midline, and those of the third, as usual, on the left side midway between these two lines. In the first group the needle went into or near the abdominal ganglion, in the second group it went into or near the heart, but in the third group it did not directly injure either of these vital organs. These tests were repeated with 30 *Phormia* males 9 days old. From the data in table 7 it is clear that the nearer the needle approached the ventral ganglion the more effective was the nicotine.

The foregoing tests were repeated by injecting 10 *Phormia* females in the mesothorax on the ventral midline. The reaction times were slightly less than those given in table 7 for females injected in the

abdomen on the ventral midline. Distilled water, used as a control, caused no signs of paralysis.

Other flies, which had been injected on the left side the previous day, were injected a second time in the wounds, which were well healed. This time the reaction times were slightly increased.

TABLE 7.—*Effect of point of injection on toxicity of nicotine to Phormia flies, as determined by the paralysis method*

Point of injection	First-reaction time			Revival time		
	Females	Males	Average	Females	Males	Average
	Minutes	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Minutes*
Dorsal midline	20.0	14.7	17.3	64.8	40.8	52.8
Left side	23.5	24.7	24.1	87.7	70.8	79.2
Ventral midline	31.0	32.6	31.8	114.2	118.7	116.4

SUMMARY

To inject minimum quantitative doses of nicotine into insects, a micrometer syringe and other devices to accompany it were used for the first time for administering insecticides. This apparatus was extremely accurate. With a minimum dose of 1.24 mm^3 the error never exceeded 4 percent and was usually less.

The comparative susceptibility to nicotine of various species and the sexes has been found to be of the following descending order: *Lucilia* male, *Lucilia* female, codling moth larva, *Calliphora* male and female, silkworm, armyworm, and *Phormia* larva. The *Lucilia* male flies were nine times as susceptible as the *Phormia* larvae. In general, the susceptibility of the larval forms was about half that of the imaginal forms.

The age of the injected flies greatly affected their susceptibility to nicotine. From the first to the fourth or fifth day susceptibility rapidly decreased; thereafter it less rapidly increased. Flies 14 days old were eight times as susceptible as flies 5 days old. The *Phormia* male flies were always more susceptible than the *Phormia* females of the same age.

It was shown statistically that solutions of free nicotine were from 22 to 26 percent more toxic than solutions of nicotine sulphate containing the same percentage of nicotine. However, when the nicotine in the nicotine sulphate solution was freed by the use of potassium hydroxide, its toxicity was equal to that of the other free nicotine.

In a study of motor paralysis old *Phormia* flies were more easily paralyzed than young flies and the paralysis lasted longer. For flies differing little in age the reaction times to increasing doses of nicotine can be represented by ascending straight lines. Males of *Calliphora* were more susceptible than females.

The first symptom of nicotine poisoning was stimulation, and this was followed by depression.

The toxicity increased as the point of injection approached the ventral ganglion.

LITERATURE CITED

(1) HOCKENYOS, G., and LILLY, J.
1932. TOXICITY STUDIES BY HYPODERMIC INJECTION OF CELERIO LINEATA LARVAE. Jour. Econ. Ent. 25: 253–261, illus.

(2) LEVINE, N. D., and RICHARDSON, C. H.
1934. THE SYNERGISTIC EFFECT OF THE CHLORIDES AND BICARBONATES OF POTASSIUM AND SODIUM UPON THE PARALYTIC ACTION OF NICOTINE IN THE COCKROACH. Jour. Econ. Ent. 27: 1170–1175, illus.

(3) MCINDOO, N. E.
1916. EFFECTS OF NICOTINE AS AN INSECTICIDE. Jour. Agr. Research 7: 89–122, illus.

(4) ———
1933. OLFACTORY RESPONSES OF BLOWFLIES, WITH AND WITHOUT ANTENNAE, IN A WOODEN OLFACTOMETER. Jour. Agr. Research 46: 607–625, illus.

(5) NOLAN, W. J.
1932. BREEDING THE HONEYBEE UNDER CONTROLLED CONDITIONS. U. S. Dept. Agr., Tech. Bull. 326, 50 pp., illus.

(6) STOREY, H. H.
1933. INVESTIGATIONS OF THE MECHANISM OF THE TRANSMISSION OF PLANT VIRUSES BY INSECT VECTORS.—I. Roy. Soc. [London] Proc., Ser. B, 113: 463–485, illus.

(7) TREVAN, J. W.
1922. AN APPARATUS FOR THE MEASUREMENT OF SMALL QUANTITIES OF FLUID. Lancet [London] 202 (v. 1 for 1922): 786, illus.

43914—38——4

RESISTANCE OF SHEEP OF DIFFERENT BREEDS TO INFESTATION BY OSTERTAGIA CIRCUMCINCTA[1]

By M. A. STEWART, *assistant entomologist*, R. F. MILLER, *associate animal husbandman*, and J. R. DOUGLAS, *research assistant in entomology, California Agricultural Experiment Station* [2]

INTRODUCTION

On August 29, 1935, 29 lambs, representing 5 breeds and 1 group of cross-breds, were separated from the general flock of sheep on the university farm at Davis, Calif., and used in an attempt to determine whether or not breed resistance to *Ostertagia circumcincta* (Stad.) existed in the breeds at hand. The breeds and birth dates of these animals are given in table 1.

TABLE 1.—*Breeds, brand numbers, and birth dates of lambs used to determine breed resistance to Ostertagia circumcincta*

Breed	Brand No.	Date of birth	Breed	Brand No.	Date of birth
Rambouillet	1	Dec. 1, 1934	Southdown	20	Jan. 31, 1935
	5	Dec. 10, 1934		19	Feb. 16, 1935
	3	Dec. 22, 1934		17	Mar. 7, 1935
	2	Dec. 24, 1934		18	Mar. 12, 1935
	4	Dec. 28, 1934		21	Mar. 16, 1935
Hampshire	26	Jan. 8, 1935	Romney	8	Feb. 7, 1935
	22	Feb. 21, 1935		9	Feb. 22, 1935
	25	Mar. 6, 1935		6	Feb. 24, 1935
	24	Mar. 9, 1935		10	Mar. 2, 1935
	23	Mar. 19, 1935		7	Mar. 15, 1935
Shropshire	12	Dec. 30, 1934	Cross-bred	27	Mar. 19, 1935
	16	Feb. 13, 1935	Shropshire-Merino	28	Mar. 20, 1935
	11	Mar. 1, 1935	Romney-Shropshire	29	Mar. 15, 1935
	13	Mar. 2, 1935			
	15	Mar. 15, 1935			
	14	Mar. 17, 1935			

Up to the beginning of the experiment these lambs were raised in the manner customary at the university farm. At the age of 2 weeks all were docked and the males were castrated. They were then pastured on native grass until about May 1, 1935, at which time they were turned into a field of Ladino clover. This field was divided into two pastures and was irrigated every 10 or 12 days. During irrigation the sheep were moved from one pasture to the other. Throughout the entire period the animals were brought to the barn every morning and fed hay and grain. They did not thrive well, however, and there was considerable scouring, which was first noticed about June 1.

All the lambs were weaned by June 15 and they were continued on Ladino clover with a grain ration once a day. On July 5 a diagnosis of coccidiosis was made in several lambs and the entire flock was treated daily for 5 days with bismuth and tannic acid in mineral oil

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[2] The authors desire to express their deep appreciation to Dr. Paul W. Gregory for advice and assistance in connection with the statistical analyses, to Dr. E. B. Roessler for checking the mathematical procedure, and to Dr. George H. Hart for criticisms and suggestions.

and fed exclusively upon dry hay. Some improvement was noted, but on July 18 a sick lamb was autopsied and a considerable infestation of *Ostertagia circumcincta* was discovered. The entire flock was treated on July 22, and again on August 5, with 2 ounces of a 1-percent solution of copper sulphate. The animals were fed hay and some grain and allowed to graze to a limited extent on Ladino clover from July 22 to August 29, when the experimental work began, and they manifested a gradually improved condition.

On August 29 all of the selected lambs with the exception of the Hampshires, were returned to Ladino clover pastures and kept there exclusively throughout the experiment. The Hampshire lambs were placed on this pasture September 6. Irrigation every 10 or 12 days was discontinued during the winter season when the pastures remained naturally moist. By September 20 the lambs appeared to be more thrifty and in better condition than for several months.

METHOD OF PROCEDURE

A fecal sample was obtained from each animal, and each lamb was weighed regularly every 2 weeks.

Fecal samples were collected by manual manipulation and the hands were thoroughly washed after each sampling to prevent contamination of succeeding samples. Each sample was wrapped in a piece of glazed onionskin paper bearing a number corresponding to the eartag. The samples were then taken to the laboratory and egg counts were made within 24 hours after the samples were collected.

The technique employed in examining the feces for eggs was a slight modification of Caldwell's method and was as follows:

(1) 1 g of feces was weighed out and placed in the bottom of a 30-ml shell vial calibrated at 10 ml.

(2) 2 ml of 30-percent antiformin was added to the feces in the shell vial and mixed thoroughly by means of a clean glass rod. The glass rod was left in the vial and the sample was allowed to stand at room temperature for at least 1 hour.

(3) Sugar solution with a specific gravity of 1.23 was added to the 10-ml mark on the vial.

(4) The glass rod was removed after stirring and the specimen was "bubbled" by means of blowing through a pipette placed against the bottom of the vial.

(5) 0.1 ml of the prepared specimen was taken from the vial with a pipette and spread on a slide so as to form a thin rectangular film.

(6) The eggs were counted with the aid of a mechanical stage under the 32-mm objective of the microscope. The number of eggs found in each smear was multiplied by 100 to express the number of eggs per gram of feces.

This slight modification of Caldwell's method was made to enable the writers to deal with smaller quantities of fecal material. Two slides of each specimen were prepared and counted for ova and the results were averaged to reduce error. The physical consistency of each specimen at the time of weighing in the laboratory was taken into consideration. A relationship of 1:2:4 is considered to exist between the formed, mushy (unformed), and diarrheic feces.

EXPERIMENTAL DATA

Figure 1 shows the average infestation, based upon the number of eggs per gram of feces, of each of the six groups of lambs and the average for the composite band from September 6, 1935, to September 4,

1936. Since the entire band was kept under identical conditions and handled in exactly the same way throughout the investigation, the egg count may be considered as a measure of resistance or susceptibility, and it is commonly accepted as such regardless of the true nature of the resistance. Assuming, then, that the egg count indicates the degree of infestation in each group, figure 1 shows an ascending order of susceptibility as follows: Romney, cross-breds, Southdown, Rambouillet, Shropshire, and Hampshire. Details of this variation in resistance or susceptibility, following exactly the egg counts made at intervals of 2 weeks, are shown in figure 2. The arrangement of the curves for the various breeds is such that in any given section of the abscissa the egg count shown for each breed was not only obtained from animals maintained under identical climatic and food conditions

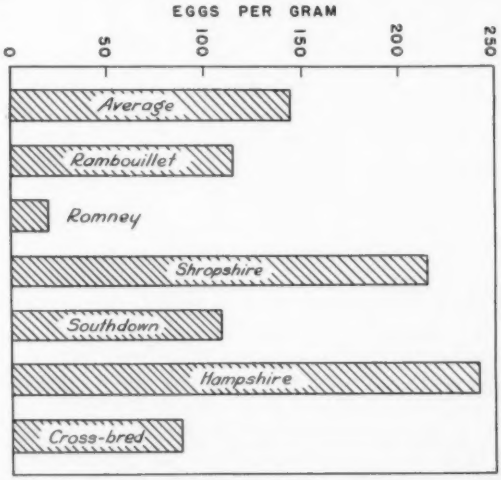

FIGURE 1.—Average infestation of *Ostertagia circumcincta* in the breeds of sheep investigated, and the average infestation for the entire band.

but from animals of practically the same age. Thus there is eliminated any possible confusion consequent upon making a comparison based upon age immunity or acquired immunity rather than upon natural resistance irrespective of age or degree of exposure to infection. It will be observed that figure 2 is in agreement with figure 1 with such minor exceptions as might be expected from a comparison of detailed data with averaged data. The peak attained by the Hampshires at 410 days was due very largely to an unusually heavy infestation in a single animal.

In order to determine whether or not such differences in resistance were significant, especially in view of the fact that a small number of each breed was under investigation, the data were analyzed by means of Fisher's pairing method.[3] The results are shown in table 2.

[3] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, 307 pp., illus. Edinburgh and London. 1932. See pp. 111–114.

TABLE 2.—*Results of analyzing the data regarding infestation by means of Fisher's pairing method in order to make comparisons between breeds*

Breeds compared	Sheep and mean egg count per gram of feces	*t* value[1]
Rambouillet with Southdown	Rambouillet 115, Southdown, 109	2.0109
Rambouillet with Hampshire	Hampshire 242, Rambouillet, 115	2.8891
Rambouillet with cross-bred	Rambouillet 115, cross-bred, 88	1.3614
Rambouillet with Romney	Rambouillet 115, Romney, 20	3.5854
Rambouillet with Shropshire	Shropshire 215, Rambouillet, 115	2.1824
Romney with Shropshire	Shropshire 215, Romney 20	4.3232
Romney with Southdown	Southdown 109, Romney 20	2.8008
Romney with Hampshire	Hampshire 242, Romney 20	3.5277
Romney with cross-bred	Cross-bred 88, Romney 20	2.9670
Shropshire with Southdown	Shropshire 215, Southdown 109	2.1200
Shropshire with Hampshire	Hampshire 242, Shropshire 215	.3783
Shropshire with cross-bred	Shropshire 215, cross-bred 88	2.6708
Southdown with Hampshire	Hampshire 242, Southdown 108	2.0577
Southdown with cross-bred	Southdown 109, cross-bred 88	.7171
Hampshire with cross-bred	Hampshire 242, cross-bred 88	3.0372

[1] The *t* value necessary to indicate significance is 2.479; high significance, 2.779.

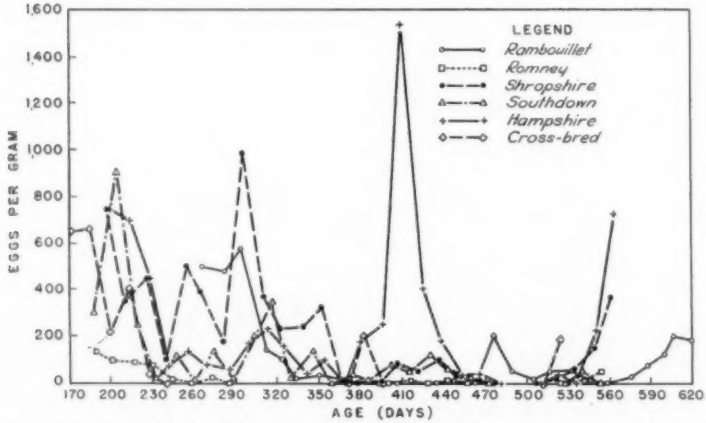

FIGURE 2.—Exact biweekly *Osterlagia circumcincta* egg counts over a 12-month period in different breeds of sheep.

It should be mentioned here that the calculations shown in table 2 are based upon the pairing of data obtained at each time of fecal sampling rather than upon mean egg counts for the entire period of investigation. It should also be pointed out that the *t* values, which are measurements of the significances of differences, where Southdowns and cross-breds are concerned are not based upon as many records as are the *t* values for other breeds, since out of five initial Southdowns, two died (one on March 11, 1936, and one on June 12, 1936), and out of only three initial cross-breds, one died on October 21, 1935. The cross-breds, however, may well be ignored since they do not represent a typical breed and are not considered further in this paper.

Extending the statistical analyses to include a study of possible significant differences in resistance between individual members of a single breed as well as differences between breeds, the results shown in table 3 were obtained.

TABLE 3.—*Results of analyzing the data regarding infestation by means of Fisher's pairing method in order to make comparisons between individuals*

Individual sheep compared	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant	Individual sheep compared	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant
Rambouillet 2 with 1	2.125	2.508	2.819	Shropshire 13 with 15	.359	2.518	2.831
Rambouillet 3 with 1	4.119	2.485	2.787	Shropshire 13 with 16	2.018	2.508	2.819
Rambouillet 4 with 1	1.795	2.492	2.797	Shropshire 14 with 15	1.458	2.583	2.921
Rambouillet 5 with 1	2.075	2.485	2.787	Shropshire 14 with 16	2.518	2.567	2.898
Rambouillet 3 with 2	1.851	2.500	2.807	Shropshire 15 with 16	3.312	2.539	2.861
Rambouillet 2 with 4	2.593	2.508	2.819	Southdown 17 with 18	.371	2.681	3.055
Rambouillett 5 with 2	.059	2.500	2.807	Southdown 17 with 19	3.164	2.528	2.845
Rambouillet 3 with 4	4.091	2.485	2.787	Southdown 17 with 20	1.741	2.624	2.977
Rambouillet 3 with 5	3.011	2.479	2.779	Southdown 17 with 21	2.759	2.539	2.861
Rambouillet 5 with 4	1.030	2.485	2.787	Southdown 18 with 19	1.403	2.681	3.055
Romney 8 with 7	.657	2.479	2.779	Southdown 18 with 20	.847	2.718	3.106
Romney 9 with 7	.148	2.479	2.779	Southdown 18 with 21	1.331	2.718	3.106
Romney 7 with 10	1.360	2.500	2.807	Southdown 20 with 19	3.181	2.583	2.921
Romney 8 with 9	1.426	2.508	2.819	Southdown 21 with 19	.989	2.508	2.819
Romney 8 with 10	2.088	2.528	2.845	Southdown 20 with 21	2.778	2.624	2.977
Romney 9 with 10	2.017	2.500	2.807	Hampshire 22 with 23	1.428	2.583	2.921
Shropshire 11 with 12	1.993	2.528	2.845	Hampshire 24 with 22	1.264	2.500	2.807
Shropshire 11 with 13	1.770	2.508	2.819	Hampshire 25 with 22	2.454	2.500	2.807
Shropshire 11 with 14	1.218	2.567	2.898	Hampshire 22 with 26	1.335	2.508	2.819
Shropshire 11 with 15	1.659	2.552	2.878	Hampshire 24 with 23	2.876	2.552	2.878
Shropshire 11 with 16	2.157	2.528	2.845	Hampshire 25 with 23	4.163	2.552	2.878
Shropshire 13 with 12	1.808	2.508	2.819	Hampshire 26 with 23	.979	2.567	2.898
Shropshire 14 with 12	2.465	2.539	2.861	Hampshire 24 with 25	.048	2.485	2.787
Shropshire 15 with 12	2.592	2.552	2.878	Hampshire 24 with 26	1.580	2.485	2.787
Shropshire 12 with 16	.702	2.528	2.845	Hampshire 25 with 26	4.263	2.492	2.797
Shropshire 14 with 13	.990	2.518	2.831				

In the Rambouillets three pairings showed highly significant, one pairing significant, and the remaining six pairings showed insignificant differences in susceptibility to *Ostertagia circumcincta*. The animal which showed a significant difference also showed a highly significant difference upon pairing with another animal. In the Romneys all pairings, six in number, showed no significant differences in susceptibility. In the Shropshires 2 pairings of a total of 15 showed significant differences in susceptibility—1 significant, the other highly significant. In the Southdowns a total of 10 pairings was made of which 2 were significant and 2 were highly significant; 1 animal being significantly resistant in 2 pairings and another being highly significantly resistant in 2. In the Hampshires one pairing was significant, two were highly significant, and the remaining seven were not significant. The animal showing a significant difference was also, in another pairing, one of those showing highly significant differences.

Having thus established not only a significant difference in susceptibility to *Ostertagia circumcincta* among different breeds but also among different individuals of the more susceptible breeds, the writers compared the more resistant individuals of each of the more susceptible breeds, again employing Fisher's pairing method, with the mean of the Romney breed. The results obtained are shown in table 4.

TABLE 4.—*Results of analyzing the data regarding infestation by means of Fisher's pairing method in order to make comparisons between the more resistant individuals of each of the more susceptible breeds with the mean of the Romney breed*

Breed and sheep no. compared with Romney	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant	Breed and sheep no. compared with Romney	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant
Rambouillet 1	0.965	2.485	2.787	Southdown 19	1.510	2.492	2.797
Rambouillet 4	1.792	2.485	2.787	Southdown 21	.263	2.500	2.807
Rambouillet 5	2.758	2.479	2.779	Hampshire 23	.919	2.552	2.878
Shropshire 12	2.194	2.508	2.819	Hampshire 26	2.454	2.485	2.787
Shropshire 16	1.158	2.500	2.807				

Of the three Rambouillets showing a significant degree of resistance only one showed a significant difference, which was nearly highly significant, from the Romney mean, indicating that two of the three animals possess a degree of resistance of approximately the same magnitude as that obtaining in the Romneys, the most resistant of the breeds studied. Of the two more resistant Shropshires both showed insignificant *t* values when paired with the Romneys. Of the two more resistant Southdowns both showed no significant difference when compared with the Romney mean. Of the two Hampshires showing significant and highly significant *t* values neither showed differences from the Romney mean which were significant.

CONCLUSIONS

From figure 1 it appears that the breeds of sheep used in this investigation vary in susceptibility to infection with *Ostertagia circumcincta* in the following ascending order: Romney, cross-bred, Southdown, Rambouillet, Shropshire, and Hampshire. The Romneys showed very many fewer eggs per gram of feces throughout a period of 12 months than the average for all the breeds, and an even greater difference is apparent when comparisons are made with the Shropshires and Hampshires. The cross-breds, Southdowns, and Rambouillets approximate one another in egg count, occupying a position about midway between the Romneys and the Shropshires and Hampshires, which approximate one another. The picture presented by this chart of average infestations is substantiated on the whole by figure 2, allowing for certain discrepancies which are to be expected when averages of data are compared with detailed data. Statistical analysis of detailed data, as presented in table 2, shows, however, that figure 1 does not give an entirely true picture. This analysis shows the Romneys to be highly significantly more resistant than any of the other breeds; the cross-breds, which may be ignored because they do not represent a true type, are significantly more resistant than the Shropshires and highly significantly more so than the Hampshires, but not significantly more resistant than any of the other breeds; the Rambouillets are highly significantly more resistant than the Hampshires but not significantly more resistant than any other breed; the Southdowns, Shropshires, and Hampshires show no significant differences in resistance or susceptibility from one another and are not significantly more resistant than any other breed. This informa-

tion forces the writers to conclude that the correct order of ascending susceptibility is as follows: Romney, cross-bred, Rambouillet, Southdown, Shropshire, and Hampshire, the last three breeds possessing a susceptibility of approximately the same magnitude. Figure 2 and unpublished results of investigations of seasonal incidence of *Ostertagia circumcincta* infestations in these and other animals on irrigated pastures indicate that age immunity or acquired immunity do not confuse this picture of breed resistance. One might reasonably expect Romneys to be more resistant to internal parasites favored by moist conditions than most if not all other breeds of sheep since they were developed in the Kent marshes in England and were held under relatively concentrated conditions. Natural selection and selective breeding, even though not scientifically conducted, would strongly tend to make such resistance characteristic of the breed.

Further statistical analyses demonstrated that certain individual animals of the susceptible, as well as the intermediate, breeds possess a resistance to *Ostertagia circumcincta* of approximately the same magnitude as the mean of that of the Romneys. It would appear, therefore, that the factor, or factors, which determine resistance have become fixed in the Romneys and that this same factor, or factors, is also in the other breeds although not fixed as in the resistant breed. Consequently it may be assumed that if a sheep breeder is desirous of securing a breed resistant to this stomach worm he may do so by careful selection within any of the above-mentioned breeds, and probably within those not investigated as well, without the necessity of attempting to cross-breed with all of its attendant difficulties and with the possible sacrifice of other and desirable characteristics typical of certain of these breeds. A similar thing has been done by Ackert and his associates [4] at Kansas State College, where breed resistance in fowls to *Ascaridia* has been demonstrated and a strain of White Leghorns resistant to this parasite has been developed by the selection of the most resistant cockerels and pullets in the flock and carried through three generations by subsequent father-daughter and brother-sister matings.

The findings given herein demonstrate to be true Stoll's idea [5] that there probably exist strains and breeds of sheep which are resistant to helminths.

SUMMARY

A group of 29 lambs, representing 5 breeds, and 3 cross-bred individuals, was isolated and kept for 12 months on an irrigated pasture, where they were continuously exposed to infection from *Ostertagia circumcincta*. Egg counts, by a modification of Caldwell's method, were made regularly every 2 weeks throughout the period. Such counts were used as an indicator to determine relative resistance or susceptibility. The data thus obtained were statistically analyzed by Fisher's pairing method.

From these analyses it was shown that the different breeds vary in susceptibility to the parasite in the following ascending order: Romney, Rambouillet, Southdown, Shropshire, and Hampshire, the last three

[4] ACKERT, J. E. PARASITES OF POULTRY. Jour. Amer. Vet. Med. Assoc. (n. s. 43) 90: 351-352. 1937.
[5] STOLL, N. R. CERTAIN NET EFFECTS IN HELMINTHIC PARASITISM, WITH SPECIAL REFERENCE TO THE SHEEP HOST. Cornell Vet. 26: 171-179. 1936.

breeds possessing a susceptibility of approximately the same magnitude. The Romneys are outstandingly resistant.

Further statistical analysis showed that in each of the more susceptible breeds there are significant differences in susceptibility, but in the Romneys there are no significant differences between individuals. Still further analysis demonstrated that the great majority of the more resistant individuals of the more susceptible breeds possess a resistance of approximately the same magnitude as the mean of that of the Romney. Since this is true it is theoretically possible for a breeder to develop a resistant strain within any of the more susceptible breeds investigated without running the risk of sacrificing a characteristic of some particular breed which he wants to maintain.

GROWTH SUBSTANCES IN RELATION TO THE MECHANISM OF THE ACTION OF RADIATION ON PLANTS [1]

By H. W. POPP, *professor of botany*, and H. R. C. MCILVAINE, *research assistant*, *Pennsylvania Agricultural Experiment Station*

INTRODUCTION

That radiation exerts a strong formative influence on plants has long been recognized. The blue-violet end of the spectrum is particularly effective in this respect, as has been shown by the work of Schanz (*7*),[2] Popp (*4*), Shirley (*8*), and others (*5*). With most plants, this part of the spectrum causes decreased stature as compared with the red end. In all the work with ultraviolet radiation carried out by the senior author since 1921 and in many of the researches of other investigators, no effect of such radiation, except perhaps the destructive action of the extremely short wave lengths, has been more consistently found than the decreased stature of plants (*6*). An exposure of as little as 2 minutes per day, to the unscreened radiation from a quartz mercury-vapor lamp at a distance of 50 cm exerts a marked stunting effect on seedlings otherwise kept in diffused daylight or in darkness.

No satisfactory explanation of the mechanism of this action of radiation has as yet been advanced. Previous attempts in the writers' laboratories to explain it on the basis of changes in such chemical substances as occur in sufficient quantities to be analyzed chemically have so far proved unsuccessful, although investigations in this field have hardly attained a good start as yet. Hare and Kersten (*3*), as a result of their discovery that ultraviolet radiation destroys indole-3-n-propionic acid, have recently suggested that "a possible action of ultraviolet light upon the tryptophan within the plant may in some part explain the effect of ultraviolet light upon plants." This inference warrants further inquiry, since it has been shown that ultraviolet radiation destroys tryptophan in vitro, and that disagreeable odors such as might result from the breaking down of tryptophan and other indole derivatives have sometimes been observed when seedlings were exposed to distinctly injurious doses of ultraviolet. The recent interest in plant-growth substances or hormones, the rapidly accumulating experimental data on their characteristics and effects, and particularly the technique developed (*1*, *9*, *10*) for their quantitative determination, have furnished another means of approach to this problem.

The present investigation was undertaken for the purpose of determining whether the effects on the plant of short-wave radiation could be explained on the basis of the effect of such radiation on plant-growth substances. The work was begun in January 1936, as a part of a more extended investigation of the effects of radiation on plants which has been under way for several years. After about 3,000 tests

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[2] Reference is made by number (italic) to Literature Cited, p. 936.

had been completed, a paper appeared by Burkholder and Johnson (2) reporting a somewhat similar investigation.

In general, these men found that intact seedlings or excised tips of seedlings of *Avena* and *Zea* exposed to the radiation from a quartz mercury-vapor lamp transmitted through 5 cm of distilled water in a glass cell or through a Corning No. 986 red-purple Corex A filter of 7 mm thickness, contained less growth substance, as determined by a modified Went method, than did controls kept in darkness, the reduction in growth substance being greater with increased exposure. That the effect of the radiation was not brought about by a downward diffusion of the growth substance away from the direction of the radiation was indicated by the fact that lower sections of treated seedlings showed no commensurate increase in growth substance. Further evidence that the effect of the radiation was a direct one was the fact that irradiated blocks of agar into which growth substance had previously been allowed to diffuse from excised coleoptile tips caused less curvature in decapitated *Avena* test plants than did control blocks not irradiated.

Ultraviolet radiation alone, as transmitted through Corning No. 986 red-purple Corex A glass, caused a marked reduction in growth substance as compared with the amount in controls. The test plants were exposed to radiation through this glass for 60 and 120 minutes at an intensity of 1,888 ergs/cm^2/sec., an exposure probably great enough to cause injury to the seedlings, since this glass transmits ultraviolet radiation down to 250 mμ. Burkholder and Johnson do not indicate whether injury occurred, but the writers have never been able to irradiate plants through this glass under the conditions stated, without injuring the exposed tissues. On the other hand, tests with the blue mercury line, 4,358Å, at an intensity of 278 ergs/cm^2/sec., indicated to Burkholder and Johnson "only small and perhaps insignificant differences in growth substance as compared with the darkened controls." It is likely that in these tests the intensity of the radiation was too low and the exposure time not great enough, for, if the stunting effect of such radiation as compared with darkness is to be attributed to reduction in growth substances, we should expect a significant difference between irradiated plants and controls. In these tests, 8 seedlings were illuminated for 55 minutes, 10 for 180 minutes, and 10 for 300 minutes. The average curvatures produced by these seedlings were 11.0°, 9.5°, and 8.7°, respectively, as against 10.9° for the controls in darkness. While there was probably too small a number of seedlings used under any condition to warrant reliable inferences, these results do at least indicate a consistent decrease in growth substance with length of exposure.

When excised tips of coleoptiles were subjected to unilateral illumination, the illuminated side contained less growth substance than the shaded side, but, strangely enough, when intact coleoptiles were thus treated, although they bent toward the light, the illuminated side contained more growth substance than the darkened side. No explanation is offered for these contradictory results.

While the Burkholder and Johnson paper was probably only intended as a preliminary one, since few tests were made under any one set of conditions, the results do indicate that the effect of radiation of short wavelengths is to inactivate growth substance. If this is to be used in explanation of the mechanism of the action of radiation

upon plant growth, it is necessary that the conditions of radiation that produce definite formative effects be the ones that are used in the studies of growth substances. With ultraviolet radiation, for example, the intensity, spectral range, and duration of exposure should be such as to cause the ordinary stunting of growth without seriously injuring the seedlings. In the present investigation an attempt was made to satisfy these conditions.

EXPERIMENTAL PROCEDURE

The test plants used in this investigation were turnips (*Brassica rapa* L.), variety Purple Top White Globe. Individually selected seeds were germinated on cotton and filter paper in sterilized Petri dishes, 50 seeds to a culture, and kept in darkness at 25° C. except during the radiation treatments. The seeds were evenly spaced in the germinators.

The source of radiation was a mercury-vapor lamp in quartz operated on a 110-volt alternating current circuit with rectifier. The intensity of radiation from this lamp at a distance of 50 cm, without any screen, was 21.65 watts (10^{-5}/cm^2). Three types of Corning glass filters were used: Noviol 0, transmitting down to 389 mμ; G586A, transmitting the region 300–436 mμ; and red-purple Corex A, transmitting the region 250–415 mμ. The total energy transmission of these filters was measured by means of an Eppley pyrheliometer of the Kimball and Hobbs type [3] and the distances from the plants to the mercury-vapor lamp were so adjusted as to equalize the intensities. In some cases, however, the plants were exposed at shorter distances. The lengths of exposures through screens transmitting ultraviolet radiation or to the unscreened arc were such as to minimize injury but to produce stunting effects on the seedlings. These periods of time were determined by the results of several years' work with such radiation. Seedlings irradiated through Noviol 0, which eliminates all ultraviolet, were given longer exposures.

The plants were usually exposed for the first time about 24 hours after being placed in the germinators, and daily or twice a day thereafter, usually for 3½ or 7 days. During irradiation a strong current of air was kept moving over the plants to minimize heating effects, although these were negligible because of the short periods of exposure. Immediately after irradiation the plants were returned to the dark chamber. Controls consisted of plants kept under all the conditions to which the test plants were exposed except the irradiation.

Tests for growth substances were made by a slight modification of the Went *Avena* method (*9*). The test plants used for this purpose were a pure-line selection of oats (*Avena sativa* L.) made by Dr. C. F. Noll of this station and chosen because of their purity and uniformity. In the first series (table 1) the tips (1 mm) of the turnip seedlings to be tested for growth substance were transferred to blocks of 3 percent agar, 2 by 2 by 1 mm, and the growth substance allowed to diffuse into them while they were kept for 2 hours in a moist chamber. These blocks were then cut in half, one half of each block used for the tests and the other half discarded. The halves used were transferred unilaterally to *Avena* coleoptiles that had been decapitated

[3] Loaned by the Eppley Laboratory, Inc.

2 hours previously and allowed to remain in a moist chamber. About 0.5 mm of the coleoptile tip was again removed before this transfer. *Avena* coleoptiles about 30 mm in length were chosen from seedlings grown in darkness for about 5 days. During all manipulations the *Avena* seedlings were fastened with special holders and glass tubes. After the transfer of the agar blocks to the decapitated coleoptiles, the latter were returned to the dark chamber for 2 hours, at the end of which time the angle of curvature of the coleoptiles was measured directly in a shadow box. All manipulations were carried out in photographically inactive light. In the later tests (table 2) the tips of the turnip seedlings were transferred directly to the decapitated coleoptiles and the angle of curvature measured. For comparison some of the *Avena* coleoptile tips were also transferred to agar and tested for growth substance. Likewise, the agar itself was tested. All results were treated statistically and probable errors determined.

TABLE 1.—*Mean curvatures obtained with agar blocks into which growth substance had diffused from tips of turnip seedlings*

[Seedlings irradiated over a period of 7 days and given a total of 7 exposures, 24 hours apart]

Screen	Daily exposure	Tests	Mean curvature
	Minutes	*Number*	*Degrees*
Unscreened arc	5	2,232	0.52±0.008
Noviol 0	20	240	2.80± .023
Control (total darkness)	0	1,808	3.10± .012

TABLE 2.—*Mean curvatures obtained by direct transfer of tips of turnip seedlings to decapitated Avena coleoptiles*

[Seedlings irradiated over a period of 3½ days and given a total of 7 exposures, 12 hours apart]

Screen	Semi-daily exposure	Tests	Mean curvature
	Minutes	*Number*	*Degrees*
Unscreened arc	5	672	0.03±0.005
Red-purple Corex A [1]	5	240	.10± .009
Red-purple Corex A [2]	5	144	.99± .013
G586A	40	624	.70± .012
G586A	20	504	1.70± .016
Noviol 0	20	576	4.90± .017
Control (total darkness)	0	1,983	5.40± .013

[1] 16 cm from lamp.
[2] 30 cm from lamp.

RESULTS AND DISCUSSION

In table 1 are given the results obtained by transferring agar blocks to the decapitated coleoptiles. The results shown in table 2 were obtained by direct transfers of the tips of the treated turnip seedlings to the decapitated coleoptiles. The latter method proved to be the better since the authors were more concerned with relative than with absolute amounts of growth substance. The growth substance present in the *Avena* coleoptile tips, as determined by the agar method, and in the agar itself is shown in table 3.

TABLE 3.—*Growth substance present in Avena coleoptile tips and in plain agar*

[Seedlings grown in dark; 3½ days old when tested]

Item	Tests	Mean curvature
	Number	*Degrees*
Avena tips	480	3.20 ±0.010
Plain agar	336	.0044± .002

An examination of tables 1 and 2 reveals that in every case irradiated plants produced lower curvatures and hence less growth substance than did the controls kept in total darkness. Furthermore, the shorter the wave lengths to which the plants were exposed, the lower were the curvatures. Thus the lowest curvatures were obtained with plants exposed to the unscreened arc, which transmitted down to about 235 mμ; the next lowest by the plants screened with red-purple Corex A, which transmitted down to 250 mμ, and the next by the plants screened with G586A, which transmitted down to about 300mμ. Increasing the length of exposure with the G586A screen or decreasing the distance with the red-purple Corex A screen still further reduced the curvatures. The plants screened with Noviol 0, which eliminates practically all ultraviolet but transmits the entire visible spectrum, gave less curvature than the controls, indicating that, although the presence of ultraviolet radiation was much more effective in reducing growth substance, this effect of radiation is not restricted to the ultraviolet region. This is in accord with what would be expected from the fact that the seedlings screened with Noviol 0 were distinctly shorter than the controls, though much longer than the plants exposed to ultraviolet radiation. The shortest plants were uniformly those exposed to the unscreened arc.

There was, therefore, a distinct correlation between the stunting effect of the radiation used and the degree of curvature produced in *Avena* coleoptiles. Since the number of tests conducted under each condition was very large, and the differences between test plants and controls were uniformly in the same direction, these results are significant. This is further shown by the statistical analysis. If the Went method of determining growth substance is accepted as accurate, these results indicate that radiation, and particularly the short wave lengths of the spectrum, causes a reduction in the amount of growth substance present in growing tips of exposed plants. If, furthermore, we accept the postulation of Went and others, that the elongation of stems is directly controlled by growth substance, these results indicate that radiation checks elongation of stems through its action on growth substances. In other words, this formative effect of radiation may be at least partly explained on the basis of inactivation of growth substance. The discovery by Burkholder and Johnson, previously referred to, that radiation inactivated growth substance which was allowed to diffuse into agar, lends further support to this conclusion.

SUMMARY

A study has been made of the amount of growth substance present in turnip seedlings kept in total darkness as compared with the amounts present in such seedlings exposed to the radiation from an unscreened mercury-vapor lamp or to this radiation as screened through Noviol 0, G586A, or red-purple Corex A glasses. Irradiated plants uniformly contained less growth substance, as determined by the Went method, than did controls in total darkness. The shorter the wave lengths to which the plants were exposed, the greater was the reduction in growth substance. Although ultraviolet radiation was more effective in reducing the amount of growth substance present in seedlings, plants exposed to only visible radiation likewise showed a lower amount than did the controls in darkness. Since the degree of stunting of the seedlings was definitely correlated with reduction in growth substance, and since growth substance has been shown to exert a controlling influence on stem elongation, these results are believed to support the thesis that the stunting effect of radiation upon plants may be at least partly attributed to the inactivation of growth substances.

LITERATURE CITED

(1) Boysen-Jensen, P.
1936. growth hormones in plants. Transl. and rev. by G. S. Avery, Jr., and P. R. Burkholder . . . 268 pp., illus. New York.

(2) Burkholder, P. R., and Johnson, E. S.
1937. inactivation of plant growth substance by light. Smithsn. Misc. Collect. 95, no. 20, 14 pp., illus.

(3) Hare, D., and Kersten, H.
1937. effect of ultraviolet radiation on indole-3-n-propionic acid. Plant Physiol. 12: 509–518.

(4) Popp, H. W.
1926. a physiological study of the effect of light of various ranges of wave length on the growth of plants. Amer. Jour. Bot. 13: 706–736, illus.

(5) ——— and Brown, F.
1936. effects of different regions of the visible spectrum upon seed plants. *In* Duggar, B. M., editor, Biological Effects of Radiation . . . , v. 2, ch. 22, pp. 763–790. New York and London.

(6) ——— and Brown, F.
1936. the effect of ultra-violet radiation upon seed plants. *In* Duggar, B. M., editor, Biological Effects of Radiation . . . , v. 2, ch. 26, pp. 853–887. New York and London.

(7) Schanz, F.
1919. wirkungen des lichts verschiedener wellenlänge auf die pflanzen. Ber. Deut. Bot. Gesell. 37: 430–442, illus.

(8) Shirley, H. L.
1929. the influence of light intensity and light quality upon the growth of plants. Amer. Jour. Bot. 16: 354–390, illus.

(9) Went, F. W.
1928. wuchsstoff und wachstum. Rec. Trav. Bot. Néerland. 25: 1–116, illus.

(10) ———
1935. auxin, the plant growth hormone. Bot. Rev. 1: 162–182, illus.

THE CARBON METABOLISM OF FUSARIUM LYCOPERSICI ON GLUCOSE[1]

By GORDON H. PRITHAM, *graduate scholar*, and ARTHUR K. ANDERSON, *professor of physiological chemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

This paper reports the results of the fourth in a series of studies begun by Anderson in 1924, when he published on the biochemistry of *Fusarium lini* (*1*).[2] The second and third papers were from this laboratory, one in 1933, the other in 1934 (*2*, *3*); both deal with *Fusarium oxysporum*. The present investigation was undertaken to obtain data on a third *Fusarium*, which would provide a basis of comparison with the previous studies.

REVIEW OF LITERATURE

One of the most important investigations of tomato wilt is that of White (*17*), who in 1927 made a study of 24 strains of *Fusarium lycopersici* with respect to pathogenicity, influence of the hydrogen-ion concentration of the medium on the growth of the organism, products of metabolism on a glucose medium, and the toxicity of these products to tomato plants.

In a study of the hydrogen-ion concentration of the soil as related to the *Fusarium* wilt of tomato seedlings, Sherwood (*14*) found that acid soils favor wilting, but that there is no limiting degree of acidity or of alkalinity at which the disease will not develop. May (*8*) found no correlation between pH or titratable acidity of five varieties of tomatoes and resistance to *Fusarium* wilt at any stage of growth. Scott (*13*) obtained maximum growth of *Fusarium lycopersici* at pH values between 4.5 and 5.3, the organism tending to change the pH of all solutions toward a constant value of 6.0.

In a recent study of the metabolism of *Fusarium lycopersici*, Luz (*7*) observed four phases in the changes in hydrogen-ion concentration of the medium brought about by the growth of the fungus. He ascribes these changes to the formation of organic acids and to the preferential absorption of ions by the fungus. He also found that, as soon as the glucose is exhausted, the organism very rapidly utilizes the ethyl alcohol which it produces.

A considerable amount of work has been done on the biochemistry of *Fusarium lini* by Anderson (*1*), Letcher and Willaman (*6*), White and Willaman (*15*, *16*), and Reynolds (*11*). This work, together with

[1] Received for publication Mar. 12, 1937; issued February 1938. Paper No. 791 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This paper is part of a thesis presented by Gordon H. Pritham to the faculty of the Graduate School of The Pennsylvania State College, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Reference is made by number (italic) to Literature Cited, p. 948.

that of Birkinshaw, Charles, Raistrick, and Stoyle (*5*) on various species of *Fusarium*, is reviewed elsewhere (*3*). Reynolds (*10*, *12*) demonstrated in resistant flax plants a cyanogenetic glucoside, linamarin, which inhibited the growth of *F. lini*, and he postulated that the fungus may release enough hydrocyanic acid from a resistant plant to inhibit its own growth.

Anderson, Everitt, and Adams (*3*) repeated much of Anderson's earlier work, using *Fusarium oxysporum* instead of *F. lini*. They found that the metabolism of the two organisms on glucose is very similar, the principal difference being that *F. oxysporum* utilized very little of the ethyl alcohol which it produced, while *F. lini* used it to a marked degree. Anderson and Emmart (*2*) studied the effect of adding certain amino acids to the culture medium on the rate of carbon dioxide production by *F. oxysporum*. The amino acid studied had little effect on the metabolic rate.

GENERAL METHODS AND MATERIALS

Source of Fusarium lycopersici culture.—The culture of the organism used in this investigation was obtained from Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station. It was isolated from Maryland tomatoes and was known as culture M. From this culture a monosporous culture was prepared by the authors. Transfers from this culture were used in the present study.

Preparation of the medium.—The medium used in this work had the following composition:

Ammonium nitrate	1.00 g.
Magnesium sulphate	0.25 g.
Monopotassium phosphate	0.50 g.
Distilled water to make	1,000 ml

To this stock solution, sufficient glucose was added to make an approximately 2-percent solution of glucose. The exact quantity of glucose in each lot of medium was determined by the Folin-Wu (*7*) method after autoclave sterilization.

Hydrogen-ion concentration.—The pH value of all solutions was determined electrometrically. The quinhydrone electrode was used in all cases except in the study of the influence of the hydrogen-ion concentration on the growth of the fungus, when the Bailey hydrogen electrode was employed.

Determination of combustible gases.—Since a low percentage recovery of carbon was obtained in preliminary work, where the methods were the same as those used in earlier work on *Fusarium lini* (*1*) and *F. oxysporum* (*3*), it was thought that possibly the fungus might produce some gases or volatile compounds which were not absorbed during aeration of the flasks for carbon dioxide. Consequently, a combustion tube, partially filled with ignited lead chromate and wire-form cupric oxide, as recommended by Pregl (*9*), and another barium hydroxide tower were added to the aeration train, the gases being passed through the hot combustion tube and into the second barium hydroxide tower after going through the first barium hydroxide tower. A significant amount of carbon was recovered by this method. That this was not merely some carbon dioxide which had escaped absorption in the first tower was shown by interposing another barium hydroxide

tower between the first one and the combustion tube. Very little carbon dioxide was obtained in this tower.

Determination of nitrogen in the mycelium.—Since Anderson and Schutte (*4*) have shown that, in the absence of chlorides, the residue from the determination of carbon by the wet combustion method can be used satisfactorily for the determination of nitrogen by the Kjeldahl method, the residue from each carbon determination after digestion was made strongly alkaline with saturated sodium hydroxide solution, the ammonia being distilled and titrated as in the usual Kjeldahl method.

The methods for the preparation and inoculation of the culture flasks, the determination of carbon dioxide, ethyl alcohol, residual glucose, organic acids, and carbon in the mycelium are essentially the same as those used in previous work (*1*, *3*).

THE REACTION OF THE MEDIUM IN RELATION TO GROWTH

OPTIMUM, MAXIMUM, AND MINIMUM pH

The technique used in studying the relation of the reaction of the medium to growth of *Fusarium lycopersici* was similar to that employed by Anderson (*1*) in his work with *F. lini*. The results are presented in figure 1. It is evident that *F. lycopersici* is very tolerant to extremes of acidity and alkalinity, the minimum pH value for growth being 1.88 and the maximum 11.06. These results correspond well with those obtained by White (*17*) with *F. lycopersici* and with those of Anderson (*1*) with *F. lini*. Luz (*7*), however, obtained no growth of *F. lycopersici* at pH values above 8.10. The lowest pH that he tried was 3.25, at which value the fungus grew well.

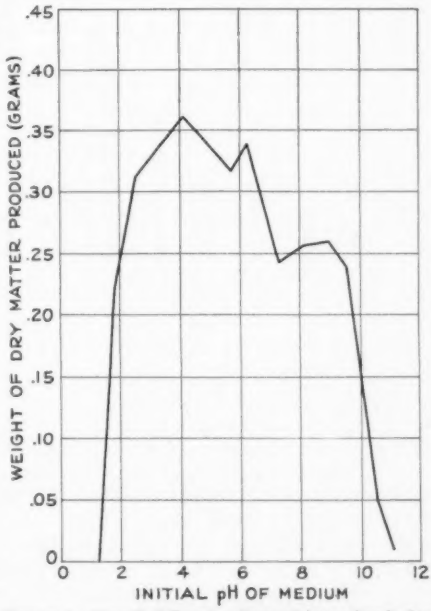

FIGURE 1.—Growth of *Fusarium lycopersici* at various hydrogen-ion concentrations.

The range for good growth is also very wide, extending from about pH 2.0 to pH 9.5, the optimum being pH 4.1. Scott (*13*) and White (*17*) obtained almost identical results with *F. lycopersici*, but observed two maxima in the growth curves at pH 4.0 to pH 5.3 and above pH 7, with a minimum between pH 5 and 7. Luz (*7*) observed maximum growth at pH 5.7. The results of the present study also agree well with those found for *F. lini* (*1*).

CHANGE IN pH DURING GROWTH

Figure 2 shows graphically the change in the reaction of the medium with time, both with and without the growth of the fungus. Perhaps the most striking effect is the marked tendency of the organism, within the range of vigorous growth, to change the reaction of the medium toward the optimum value. This effect is especially notable on the acid side, the final pH values of five cultures that had initial reactions of from 1.88 to 6.28 varying only from 4.25 to 4.95—values that are well within the optimum pH range. That these changes are due to the growth of the fungus and not to independent changes in the reaction of the medium itself is shown by the fact that the change in the reaction of the uninoculated medium tends to be constant. The

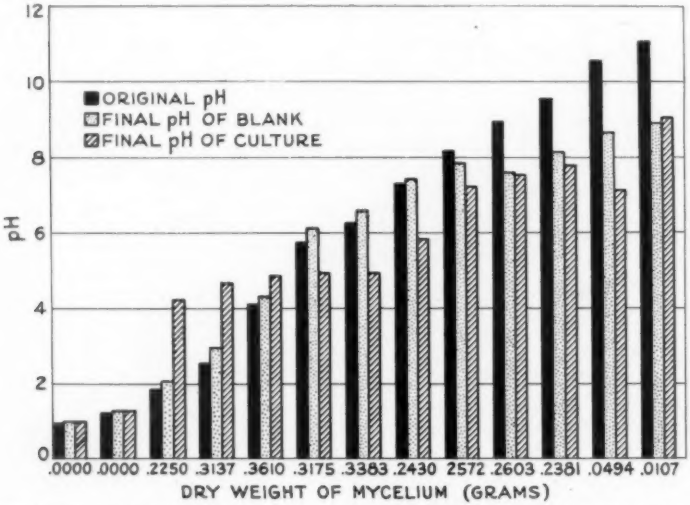

FIGURE 2.—Changes in hydrogen-ion concentration of the medium during growth of cultures shown in figure 1.

final pH of the blanks bears an approximately constant ratio to the initial pH, except in the strongly alkaline solutions, where there is evidently considerable absorption of carbon dioxide from the air. Although the change in the reaction of the inoculated medium is not so striking on the alkaline side as on the acid side of neutrality, the organism shows a decided tendency to increase the hydrogen-ion concentration toward the optimum. Part of this increase evidently is due to absorption of carbon dioxide from the air, but here again the change in the inoculated flasks is greater than in the uninoculated ones, indicating that the fungus itself changes the reaction of the medium. These results are similar to those obtained by Scott (*13*) with *Fusarium lycopersici*, and by Anderson (*1*) with *F. lini*.

In studies of the carbon metabolism on glucose, the change in pH was followed at intervals over a considerable period of growth. In all, four studies were made, and in every case the pH value of the medium was increased to about 7.0. There was no initial decrease, as Luz (7) observed in his work with *Fusarium lycopersici*, and as Anderson (1) found with *F. lini*. The pH value of an 8-months-old culture of *F. lycopersici*, which was used in testing for organic acids, was 7.3, showing that the fungus will change the reaction of the medium from acid to alkaline if allowed to grow long enough. Luz (7) ascribes the increase in pH to 7.5, as observed by him, to the preferential absorption of nitrate ions from ammonium nitrate in the medium, with the concurrent liberation of ammonium ions.

CARBON METABOLISM ON GLUCOSE

Although four studies (series) were made, the results of only the last will be presented in detail as it is representative of the other three (table 1 and fig. 3). Since in the first series the total recovery of the original carbon decreased to less than 50 percent as growth progressed, it was thought that the fungus might produce some compounds other than the ones being determined. Accordingly, two new methods were introduced in the subsequent series. It was suggested that some of the undetermined carbon might be in the form of gaseous or volatile compounds which would be lost during the aeration of the cultures for carbon dioxide. To test this hypothesis, a combustion tube and a barium hydroxide tower were introduced into the aeration train. By means of this technique, the results of which are given as "Combustion" in table 1 and figure 3, an amount of carbon representing about 2 percent of the original carbon was obtained, thus showing that a small amount of volatile organic compounds is produced by *Fusarium lycopersici*.

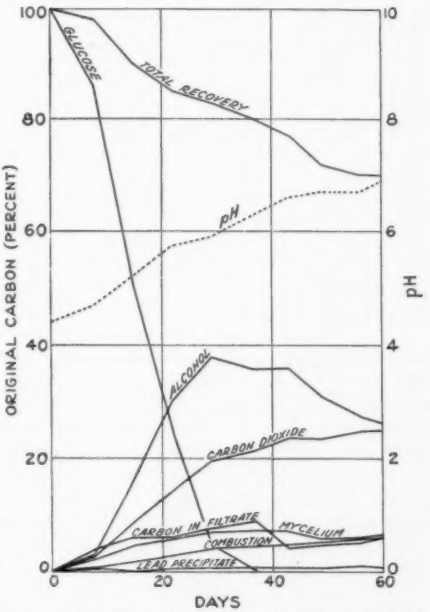

FIGURE 3.—Distribution of metabolic products of *Fusarium lycopersici* when grown on glucose. See table 1.

TABLE 1.—*Distribution of metabolic products of Fusarium lycopersici on a glucose medium at various stages of growth*

Age of culture (days)	Mycelium					Carbon dioxide		Ethyl alcohol	
	Weight	Weight of carbon	Carbon	Carbon as related to initial carbon [1]	Nitrogen	Weight of carbon	Carbon as related to initial carbon [1]	Carbon in 300 ml	Carbon as related to initial carbon [1]
	Gram	*Gram*	*Percent*	*Percent*	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*
0	0.120	0.081	45.88	2.31	3.62	0.068	2.97	0.075	3.20
8	.230	.110	47.79	4.70	3.22	.213	9.08	.374	15.96
15	.307	.140	45.62	5.98	3.33	.341	14.55	.712	30.44
22	.347	.169	48.56	7.20	4.27	.446	19.55	.890	38.01
29	.383	.177	46.26	7.58	4.03	.502	21.46	.825	35.76
37	.385	.176	45.91	7.54	4.00	.549	23.45	.841	35.96
43	.341	.143	41.63	6.12	3.68	.547	23.36	.706	30.73
49	.366	.147	40.00	6.26	3.38	.574	24.55	.644	27.32
56	.365	.156	42.67	6.65	3.62	.575	24.58	.612	26.11

Age of culture (days)	Glucose		Lead precipitate		Combustion		Residual carbon in filtrate		Total recovery of carbon as related to initial carbon [1]	pH
	Carbon in 300 ml	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]		
	Gram	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*	*Percent*	
0	2.34	100.0							100.0	4.4
8	2.01	85.8	0.010	0.42	0.024	0.70	0.06	4.00	98.0	4.7
15	1.19	50.9	.003	.12	.044	1.88	.15	6.20	90.0	5.2
22	.57	24.2	0.008	.40	.071	3.03	.15	6.20	85.0	5.8
29	.12	5.0	.020	.80	.101	4.30	.17	7.90	83.0	5.9
37	.01	.3	.023	1.00	.112	4.80	.20	8.70	80.0	6.3
43	.00	.0	.023	1.00	.119	5.07	.11	4.48	77.0	6.6
49	.00	.0	.021	.87	.128	5.35	.12	4.70	72.0	6.7
56	.00	.0	.028	1.15	.141	6.04	.13	5.30	70.0	6.7
60	.00	.0	.023	1.00	.143	6.10	.14	6.00	70.0	6.9

[1] Expressed as percentage of the carbon in the glucose originally present.

As a further check on the recovery of carbon, an aliquot of the filtrate from the original cultures was analyzed for total carbon by the wet combustion method. The sum of the carbon in the alcohol, residual glucose, and lead precipitate was then subtracted from this total carbon, giving the undetermined, or residual carbon in the filtrate. A maximum of 8.7 percent of the original carbon could be accounted for in this way, indicating that a significant amount of some organic compounds, other than ethyl alcohol, which are not precipitated by lead acetate, is formed by the organism from glucose. The curve for the total recovery of carbon in figure 3 includes both the residual carbon and the carbon obtained by combustion.

Table 1 and figure 3 show that ethyl alcohol and carbon dioxide are the main products of the metabolism of the organism on glucose, the alcohol being produced slowly at first and then more rapidly until the glucose has been exhausted, when it decreases fairly rapidly. Since carbon dioxide production contines to increase after the consumption of the glucose, it is evident that *Fusarium lycopersici* can utilize ethyl alcohol for energy and for growth. These facts are in accord with the results obtained by Anderson (*1*) with *F. lini*, except that the decrease in the alcohol, as well as the rate of metabolism for the entire series, is

not as rapid as he found it to be. Anderson, Everitt, and Adams (*3*) found that *F. oxysporum* was able to utilize ethyl alcohol as a source of carbon to only a slight extent. In this connection the recent work of Luz (*7*) should be mentioned. He found that *F. lycopersici*, when grown on a medium containing 5 percent of glucose, produced alcohol rapidly until the glucose was exhausted on the eighteenth day of growth, when the alcohol was used at a rapid rate until it was finally exhausted on the thirty-fifth day of growth. Since, in the present study, a considerable amount of alcohol was present in the medium after 60 days' growth, it is obvious that the rate of metabolism of the strain of *F. lycopersici* used by him was much greater than that of the strain employed in this investigation. However, his mineral nutrient solution was 10 times as concentrated as the one used in the present work.

The lead precipitate is significant because it is nearly constant, although representing only a small fraction of the original carbon. Evidently *Fusarium lycopersici* produces only small amounts of organic acids, the lead salts of which are insoluble. Anderson (*1*) also found that only small amounts of these acids were produced by *F. lini*.

The residual carbon in the filtrate increases rather rapidly until the glucose is practically exhausted, and then decreases. Since this decrease is at a somewhat more rapid rate than the corresponding decrease in alcohol and in the total carbon in the filtrate, it is evident that the fungus utilizes this carbon more easily than it does ethyl alcohol. As old cultures have an esterlike odor resembling that of apricots, it is possible that this residual carbon is represented by some organic acids which were not determined by any of the methods used.

The amount of volatile compounds determined by combustion shows a gradual increase during the entire growth period. The highest value was obtained in the last series at 60 days, when 6.1 percent of the original carbon was in this form. At least a part of this carbon may be in the form of volatile acids, since in subsequent experiments a small amount of these acids was found in old cultures of the organism.

The changes in the pH of the medium with growth are practically uniform in all series and the final pH is nearly the same in all series, being well within the optimum range for growth. Luz (*7*) found that he could divide the growth of *Fusarium lycopersici* into four stages on the basis of the pH changes during growth, but no such periods, or "breaks," in the pH curves were obtained in this work. Anderson (*1*) observed that *F. lini* first decreased the pH of the medium and then gradually increased it, the final value depending on the initial pH of the medium.

QUALITATIVE TESTS FOR ORGANIC ACIDS

The filtrate from an 8-months-old culture of *Fusarium lycopersici* was distilled from acid solution, and the distillate titrated with standard alkali. An amount of volatile acid equivalent to about 4.0 ml of normal acid, or 0.24 g of acetic acid, was obtained. The residue from the distillation was tested for oxalic acid by the usual precipitation as calcium oxalate. Since there was obtained a white precipitate which decolorized potassium permanganate in sulphuric acid solution, it was concluded that oxalic acid was formed in small amounts, thus confirming the findings of Luz (*7*). A doubtful test for tartaric acid was also obtained.

COMPOSITION OF MYCELIUM

The data for the percentage of carbon and of nitrogen in the mycelium, calculated on the dry weight of mycelium, are given in the column headed "Mycelium" in table 1. There is a tendency for the carbon content of the mycelium to decrease with age, a change exactly opposite to that found for *Fusarium lini* (*1*) and for *F. oxysporum* (*3*).

In general, the nitrogen in the mycelium tends to decrease slightly with age, indicating that old cultures contain less active protoplasm than fresh cultures. These results agree with those obtained for *Fusarium lini* (*1*).

Carbohydrates contain about 40 percent of carbon, proteins 51 to 55 percent, and a typical fat such as tristearin 76.87 percent. During the first 43 days of growth the carbon content of the mycelium ranged from 45.62 to 48.56 percent. During the final stages of growth the carbon content of the mycelium dropped to between 40.00 and 42.67 percent. Since the percentage of nitrogen in the mycelium decreased together with the percentage of carbon it appears that the carbohydrate content of the mycelium was increasing with age. There was no evidence of fat formation, as was the case with *Fusarium lini* (*1*) and *F. oxysporum* (*3*).

RATIO OF PRODUCTS OF METABOLISM TO ONE ANOTHER AND TO THE GLUCOSE CONSUMED

Various coefficients and equivalents have been introduced into the literature to express the relationships existing among the products of metabolism and the compounds consumed by a fungus. A number of these ratios have been calculated for each series of experiments in the present investigation; the ones for the last series are presented in table 2. To avoid confusion, the exact meaning of each ratio is given at the top of each column.

The respiration coefficient represents the grams of carbon dioxide produced per gram of dry mycelium. There is an increase in this ratio with age up to a certain point, and then a slight decrease, this point usually coinciding with the exhaustion of the glucose and the beginning of the utilization of ethyl alcohol. Evidently *Fusarium lycopersici* is able to use ethyl alcohol better for mycelium building than as a source of energy. *F. lini* (*1*) and *F. oxysporum* (*3*) showed a steady increase in this ratio.

TABLE 2.—*Quantitative relationships existing between the various metabolic products of Fusarium lycopersici when grown on a glucose medium*

Age of culture (days)	Respiration coefficient: $\frac{\text{Weight of } CO_2}{\text{Weight of mycelium}}$	Economic coefficient: $\frac{\text{Weight of sugar consumed}}{\text{weight of mycelium}}$	Respiration equivalent: $\frac{\text{Carbon of } CO_2 \times 100}{\text{carbon consumed}}$	Plastic equivalent: $\frac{\text{Carbon of mycelium} \times 100}{\text{carbon of glucose consumed}}$	Alcohol equivalent: $\frac{\text{Carbon of alcohol} \times 100}{\text{carbon of glucose consumed}}$	Carbon of alcohol / Carbon of CO_2
8	2.08	6.83	20.60	24.72	22.70	1.10
15	3.39	12.46	18.50	9.57	32.49	1.76
22	4.07	14.42	19.25	7.92	40.20	2.09
29	4.72	16.02	20.10	7.59	40.70	2.00
37	4.83	15.23	21.56	7.59	35.40	1.64
43	5.22	15.19	23.46	7.54	35.95	1.53
49	5.88	17.15	23.37	6.11	30.15	1.29
56	5.76	16.00	24.52	6.22	27.48	1.12
60	5.77	16.04	24.60	6.66	26.17	1.07

Similar changes take place in the economic coefficient, which is the amount of carbon source required to make 1 g of dry mycelium, the ratio increasing to a maximum and then decreasing as the fungus changes from glucose to alcohol as a building material. *Fusarium lini* (*1*) showed similar changes, but the maximum value was only 13.1, as compared with 17.15 in the case of *F. lycopersici*. Evidently the former organism uses carbon sources more efficiently than the latter. That young mycelium is more efficient in sugar consumption than older mycelium is illustrated by the low value obtained in every case for the first growth period, usually a week or 10 days.

As would be expected, the percentage of the carbon consumed that is transformed into carbon dioxide, or the respiration equivalent, increases with age during the growth of the fungus, since ethyl alcohol is used as a source of energy after the glucose has disappeared.

The plastic equivalent is really the reciprocal of the economic coefficient, since it represents the percentage of the glucose consumed that is converted into mycelium. As would be expected, it shows reciprocal changes, decreasing with age until the glucose is entirely used, and then increasing slightly. These changes also tend to show that ethyl alcohol is used somewhat more efficiently than glucose for mycelium production.

The alcohol equivalent is the percentage of glucose consumed that is converted into ethyl alcohol. The changes in this ratio show that *Fusarium lycopersici* utilizes ethyl alcohol after it has used all the glucose, since the values increase until the point at which the glucose is exhausted is reached, after which they decrease markedly.

In the last column of table 2 the ratio of the carbon in alcohol to the carbon in carbon dioxide is given. In a typical alcoholic fermentation the ratio of carbon in alcohol to carbon in CO_2 is 2:1. Table 2 shows that the experimentally determined ratio approaches very closely the theoretical, and is exactly 2.00 on the day of maximum alcohol production. Because of the utilization of the alcohol by the fungus, the ratio decreases to 1.07:1 on the sixtieth day of growth, indicating that about 25 percent of the alcohol is changed into carbon dioxide. The actual decrease in alcohol is over 30 percent, the difference being accounted for by the growth of the mycelium.

UTILIZATION OF ETHYL ALCOHOL

Since *Fusarium lycopersici* utilizes the ethyl alcohol which it produces from glucose, the effect on the growth of the fungus of various concentrations of ethyl alcohol, both alone and in the presence of the same concentration of glucose that was used in the preceding work, was studied. Two series of 26 flasks each were used, each flask in one series containing 100 ml of mineral nutrient medium without glucose, and each flask in the other series containing the same amount of medium with enough glucose added to make an approximately 2-percent glucose solution. The exact amount of glucose was not determined, but was assumed to be the same in all flasks containing it, since the medium for each flask was measured from one large lot. To 24 flasks from each series, amounts of 95 percent ethyl alcohol varying from 0.2 to 6.0 ml were added after sterilization. Two flasks were used as controls, no alcohol being added. After 10

days growth, the mycelium was removed by filtration and dried to constant weight at 100° C. The results are presented in figure 4.

In low concentrations, ethyl alcohol is a good source of carbon for *Fusarium lycopersici*. The fungus will grow slightly, even in concentrations as high as 4.90 g per 100 ml of solution. That maximum growth occurred at a concentration of 0.41 g per 100 ml is significant, as the maximum amount of alcohol produced by the fungus in any of the studies of the glucose metabolism was 0.57 g per 100 ml. The limiting value for maximum growth is apparently between these two figures. These results compare favorably with those of Anderson (*1*), who obtained maximum growth of *F. lini* at an alcohol concentration of 0.38 g per 100 ml. A concentration of 3.59 g per 100 ml, however, inhibited completely the growth of this organism.

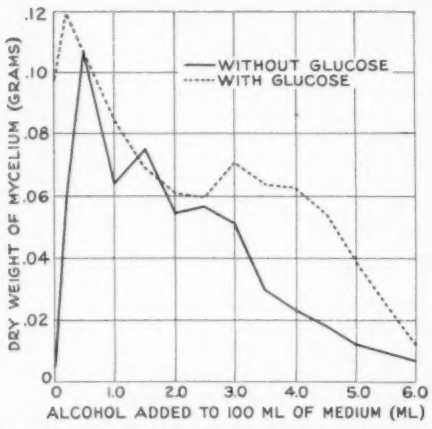

FIGURE 4.—Mycelium production on various concentrations of ethyl alcohol, with and without glucose.

As was expected, growth was generally heavier in the flasks containing glucose in addition to alcohol, although the two growth curves tend to parallel each other. That low concentrations of alcohol have a stimulating effect on the growth of the fungus on glucose is evidenced by the fact that the weight of mycelium produced on the solution containing 0.16 g of alcohol is significantly greater than that on the glucose solution alone. As the amount of alcohol increases, however, the weight of mycelium decreases until, at a concentration of 4.90 g per 100 ml, less mycelium is produced than on a solution containing 4.08 g of alcohol and no glucose.

DISCUSSION OF RESULTS

Fusarium lycopersici is as tolerant to extremes of hydrogen- and hydroxyl-ion concentration as *F. lini*, and the optimum pH value for growth is practically the same for both organisms. *F. lycopersici* is apparently better able to change the pH of strongly acidic solutions toward the optimum value than is *F. lini*. In view of the ability of the organism to grow over such a wide range of pH values, it would not appear feasible to attempt to control the *Fusarium* wilt of tomatoes by adjusting the pH of the soil, although Sherwood (*14*) found that there was a greater percentage of infection of tomato seedlings by the fungus in acid soils.

The results of the work on the carbon metabolism of *Fusarium lycopersici* on glucose indicate that this organism produces, in addition to ethyl alcohol and carbon dioxide, appreciable quantities of volatile and nonvolatile compounds, part of which was determined by new

methods. Because of the striking esterlike odor of old cultures, it is thought that fatty acids comprise an appreciable portion of these compounds, although attempts to identify these acids were unsuccessful.

The production of these compounds is an essential point of distinction between this organism and *Fusarium lini* and *F. oxysporum*. In the work with these last two fungi over 90 percent of the original carbon could be accounted for in the ethyl alcohol, carbon dioxide, and mycelium, whereas in the present study the maximum percentage of the original glucose that could be recovered in the form of these three products was less than 70 percent. This difference in the percentage recovery probably is due to actual differences in the biological characteristics of the organisms and not to faulty technique.

Another biochemical difference between the three species of *Fusaria* under discussion appears in the carbon content of old mycelia. In *Fusarium lycopersici* the carbon content of the mycelium approaches that of a carbohydrate, whereas with *F. lini* and *F. oxysporum* there is an indication of fat deposition with age.

The course of the alcoholic fermentation caused by *Fusarium lycopersici* is practically the same as that of *F. lini* and of *F. oxysporum*, alcohol and carbon dioxide being produced in approximately the same proportions by all three fungi. The amount of alcohol produced by *F. lycopersici*, however, was somewhat less than that produced by the other two organisms. The maximum quantity obtained in this work was 1.7 g, as compared with 2.1 to 2.2 g in the other investigations.

No attempts were made to discover the substances that are toxic to tomato plants. It does not seem likely, however, that the organism produces alcohol in sufficient quantity to account for its wilting effect, as the maximum concentration obtained in these studies was 0.57 g per 100 ml, and Luz (*7*) found that tomato plants were not wilted when placed in alcohol solutions containing 2 g per 100 ml. White (*17*) found that tomato cuttings were not wilted by concentrations of alcohol up to 4 percent by volume, but that the low molecular weight fatty acids and oxalic acid were highly toxic in concentrations of only 0.06 percent. Concentrations of alcohol of 4 percent or more inhibited the growth of the fungus, even in the presence of glucose, which is presumably an easily assimilable form of carbon; hence it is not likely that the concentration of alcohol in the tissues of the infected plant would become great enough to cause wilting and death of the plant.

SUMMARY

Fusarium lycopersici, the organism which causes tomato wilt, is not sensitive to extremes of hydrogen-ion and hydroxyl-ion concentration. It will grow on artificial media at pH values between 1.88 and 11.06.

The range for good growth is also wide, extending from pH 2.0 to pH 9.5.

The organism shows a decided tendency to change the pH of both acid and alkaline media toward the optimum value, the final pH in five cases varying only between pH 4.25 and pH 4.95.

The principal products of the metabolism of the organism on glucose are carbon dioxide and ethyl alcohol, but considerable quantities of other compounds, presumably volatile and nonvolatile organic acids,

are formed as well. Some of the carbon in these unknown compounds was determined by means of new procedures.

The ratio of the carbon in alcohol to carbon in carbon dioxide is very nearly that required by the equation for a typical yeast fermentation. Because of the consumption of the alcohol by the fungus during the later periods of growth, however, this ratio gradually decreases with the age of the culture.

Fusarium lycopersici definitely uses the ethyl alcohol that it produces for energy and growth as soon as the glucose in the solution has been exhausted. That it uses the alcohol somewhat more efficiently than the glucose is evidenced by the slow decrease in the plastic equivalent while the organism is using glucose and the sudden increase in this ratio when alcohol becomes the carbon source.

Ethyl alcohol will serve as the sole carbon source for *Fusarium lycopersici*, maximum growth being obtained on a concentration of alcohol approximating the maximum amount produced by the fungus on a glucose medium. Concentrations of alcohol of 4 percent by volume or more seriously inhibited the growth of the fungus. A small amount of alcohol (0.2 percent) stimulated mycelium production on a glucose medium, whereas concentrations of 5 percent by volume or more inhibited growth.

LITERATURE CITED

(1) Anderson, A. K.
1924. Biochemistry of plant diseases. The biochemistry of Fusarium lini, Bolley. Minn. Univ. Studies Biol. Sci. 5: 237–280.

(2) ——— and Emmart, K.
1934. Relation of certain amino acids to carbon dioxide and mycelium production of Fusarium oxysporum. Plant Physiol. 9: 823–829, illus.

(3) ——— Everitt, E. L., and Adams, P. D.
1933. The carbon metabolism of Fusarium oxysporum on glucose. Jour. Agr. Research 46: 473–482, illus.

(4) ——— and Schutte, H. S.
1924. The determination of nitrogen in connection with the wet combustion method for carbon. Jour. Biol. Chem. 61: 57–61.

(5) Birkinshaw, J. H., Charles, J. H. V., Raistrick, H., and Stoyle, J. A. R.
1931. Studies in the biochemistry of micro-organisms. Part V.—Quantative examination by the carbon balance sheet method of the types of products formed from glucose by species of Fusarium. Roy. Soc. [London], Phil. Trans., Ser. B, 220: 93–98.

(6) Letcher, H., and Willaman, J. J.
1926. Biochemistry of plant diseases VIII alcoholic fermentation of Fusarium lini. Phytopathology 16: 941–949, illus.

(7) Luz, G.
1934. Über den Stoffwechsel von Fusarium lycopersici und Fusarium lini. Phytopath. Ztschr. 7: [585]–638, illus.

(8) May, C.
1929. Hydrogen ion concentration and titratable acidity of tomatoes and their resistance to Fusarium wilt. Ohio Jour. Sci. 29: 260–266.

(9) Pregl, F.
1930. Quantitative organic microanalysis. English ed. 2, transl. from rev. and enl. German ed., by E. Fyleman. 237 pp., illus. London.

(10) REYNOLDS, E. S.
1924. SOME RELATIONS OF FUSARIUM LINI AND POTASSIUM CYANIDE. Amer. Jour. Bot. 11: 215–217.
(11) ———
1926. NUTRITIONAL STUDIES ON FUSARIUM LINI. Plant Physiol. 1: 151–164, illus.
(12) ———
1931. STUDIES ON THE PHYSIOLOGY OF PLANT DISEASE. Ann. Mo. Bot. Gard. 18: 57–95, illus.
(13) SCOTT, I. T.
1924. INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF FUSARIUM LYCOPERSICI AND ON TOMATO WILT. Mo. Agr. Expt. Sta. Research Bull. 64, 32 pp., illus.
(14) SHERWOOD, E. C.
1923. HYDROGEN-ION CONCENTRATION AS RELATED TO FUSARIUM WILT OF TOMATO SEEDLINGS. Amer. Jour. Bot. 10: 537–553, illus.
(15) WHITE, M. G., and WILLAMAN, J. J.
1928. BIOCHEMISTRY OF PLANT DISEASES. X. FERMENTATION OF PENTOSES BY FUSARIUM LINI. Biochem. Jour. 22: [583]–591, illus.
(16) ——— and WILLAMAN, J. J.
1928. BIOCHEMISTRY OF PLANT DISEASES. XI. FUSARIUM LINI AND THE PYRUVIC ACID THEORY OF ALCOHOL FERMENTATION. Biochem. Jour. 22: [592]–595.
(17) WHITE, R. P.
1927. STUDIES ON TOMATO WILT CAUSED BY FUSARIUM LYCOPERSICI SACC. Jour. Agr. Research 34: 197–239, illus.

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